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Applicants: Andrew VAILLANT et al.
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Filing Date: September 12, 2003
For: ANTIVIRAL OLIGONUCLEOTIDES TARGETING RSV
Art Unit: 1648
Examiner: Sharon L., HURT
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DECLARATION UNDER 37 C.F.R. SEC. 1.132

I, Jean-Marc Juteau, do hereby declare and state as follows:

1. I received the degrees of Bachelor (B.Sc.) of Biology from Montreal University in 1985, Master (M.Sc.) of Microbiology and Immunology from Montreal University in 1988, and Doctor of Philosophy (Ph.D.) of Microbiology and Immunology from Laval University in 1991.
2. My academic background and experiences in the field of the present invention are listed on the enclosed *curriculum vitae*.
3. I am a founder since 1999 of REPLICor Inc. and Senior Vice President since 2002.
4. I am an author of several scholarly publications as listed in my enclosed *curriculum vitae*.

5. I am an inventor in the present application; I have read and am thoroughly familiar with the contents of U.S. Patent Application Serial No. 10/661,415 entitled "ANTIVIRAL OLIGONUCLEOTIDES TARGETING RSV", including the claims.
6. I have also read and understood the latest Official Action from the PTO dated April 28, 2006. In this Office Action, claims 1-2, 14-32 and 38 were rejected for lack of enablement under 35 U.S.C. §112, first paragraph.
7. The following experiment had been performed in Nov. 2004 for the respiratory syncytial virus (RSV) cotton rat model under the supervision of Andrew Vaillant (inventor on this invention) and myself, to obtained results in an animal model of RSV infection showing the *in vivo* antiviral activity occurring by a non-sequence complementary mode of action of oligonucleotides of the present invention. RSV is a member of the *Paramyxoviridae* as for the parainfluenza virus. This *in vivo* cotton rat model of RSV infection is a recognized predictive model that can be used for the demonstration of a drug treatment activity.
8. The following experiments were conducted to evaluate the therapeutic antiviral activity of sequence independent oligonucleotides *in vivo*.

Respiratory syncytial virus

RSV infection in the cotton rat is an animal model which has been predictive of antiviral activity against respiratory syncytial virus (RSV) infections in humans. The model is widely accepted for testing the activity of compounds *in vivo* (Maggon & Barik, 2004, Rev, Med Virol. 14:149-168; Sidwell & Barnard, 2006,

Antiviral Res. 71:379-390) While the progression of infection in this model is slower than in mice (or in humans), it has been demonstrated that the viral receptor utilization by RSV in the cotton rat is highly similar to that in humans.

A phosphorothioated 40mer randomer (REP 2006) was administered daily to cotton rats by aerosol inhalation starting 1 day prior to infection with a human strain of RSV (rat adapted) and continuing for 2 days after infection. REP 2006 was prepared as a 100 mg/ml solution of the sodium salt of REP 2006 into water. The solution was heated to 65°C for 15 minutes, and then allowed to cool to room temperature. Cooled solutions were then filter sterilized using a 0.22um cellulose acetate filter. Solutions were stored at -20°C until use. Samples were thawed to room temperature and then heated to 37°C for 5 minutes before administration. REP 2006 solution was added to the reservoir of a Acrotech II nebulizer and was then used to generate REP 2006 aerosol using air delivered to the nebulizer at a flow rate of 10 L/min @ 15 PSI. REP 2006 aerosol was directed using standard medical aerosol tubing into the cage where the mice were situated. Aerosol exposure was allowed to continue for 30 min which used approximately 10 ml of REP 2006 solution. Antiviral activity assessed by the determination of viral titers in the lungs, is reported in Table 1.

Table 1
Effect of aerosol administration of ONs in RSV infected cotton rats.

REP 2006 dose and route of administration	RSV lung titer (log ₁₀ /g lung) 4 days after infection
0 (placebo control)	3.9
2X100mg/ml/day (10ml aerosol) 24h after infection	2.8

These results show that oligonucleotides of this invention are an effective and significant *in vivo* treatment against an *Paramyxoviridae* infection such as RSV. The administration of oligonucleotides of the present invention was well tolerated in this model.

9. The results presented hereinabove and produced according to the teaching disclosed in the U.S. Patent Application Serial No 10/661,415, clearly proves that that the present invention has clinical relevance and in addition, that the *in vitro* results disclosed in the present application do not diverge from *in vivo* responses. The antiviral activity occurring by a non-complementary mode of action of oligonucleotides of the present invention is demonstrated *in vivo* in a predictive model of RSV infections.

10. I hereby declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true, and that these statements were made with the knowledge that willful false statements and the like so made are punishable by a fine or imprisonment, or both (18 U.S.C. Sec. 1001), and may jeopardize the validity of the application of any patent issuing thereon.

Signed



Jean-Marc Juteau

Dated: Sept. 13, 2006

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Biopharmaceutical company developing antiviral and anticancer drugs.

Responsibilities:

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Day to day contact with CSO, scientific input.
- In charge of Intellectual property portfolio.
Patent writing, strategy, management.

02-1999 – 01-2002

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Responsibilities:

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- In charge of financing
Instrumental in raising \$2.5M in equity and loan
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License, research, option, confidentiality, material distribution.
- Spin-off company projects
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Product Manager, Iso Tech Design, Laval
Company developing and marketing micro-environments for pharma applications.

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Molecular biology, epidemiology and structure-function analysis of the ROB-1 β -lactamase.

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Bachelor (B.Sc.), Biology, **Montreal University**.

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2004- today

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Medical Research Council (MRC) Fellowship, 1992.
Fonds de la Recherche en Santé du Québec (FRSQ) Studentship, 1989-90-91.
Fonds pour la Formation des Chercheurs et l'Aide à la Recherche (FCAR) Studentship, 1988-89.
Canlab Prize from l'Association des Microbiologistes du Québec, 1989.

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AUTHORSHIP

Patent filings: 20
Scientific articles: 10
Posters and oral presentations: 30

Vaillant A, Juteau JM, Lu H, Liu S, Lackman-Smith C, Ptak R, Jiang S. Phosphorothioate oligonucleotides inhibit human immunodeficiency virus type 1 fusion by blocking gp41 core formation. *Antimicrob Agents Chemother*. 2006 Apr;50(4):1393-401.

Kocisko DA, Vaillant A, Lee KS, Arnold KM, Bertholet N, Race RE, Olsen EA, Juteau JM, Caughey B. Potent antiscrapie activities of degenerate phosphorothioate oligonucleotides. *Antimicrob Agents Chemother*. 2006 Mar;50(3):1034-44.

Moaddel R, Price GB, Juteau JM, Leffak M, Wainer IW. The synthesis and initial characterization of an immobilized DNA unwinding element binding (DUE-B) protein chromatographic stationary phase. *J Chromatogr B Analyt Technol Biomed Life Sci*. 2005 Jun 25;820(2):197-203.

Sylvestre M, Sirois M, Hurtubise Y, Bergeron J, Ahmad D, Shareck F, Barriault D, Guillemette I, Juteau JM. Sequencing of *Comamonas testosteroni* strain B-356-biphenyl/chlorobiphenyl dioxygenase genes: evolutionary relationships among Gram-negative bacterial biphenyl dioxygenases. *Gene*. 1996 Oct 3;174(2):195-202.

Ahmad D, Fraser J, Sylvestre M, Larose A, Khan A, Bergeron J, Juteau JM, Sondossi M. Sequence of the bphD gene encoding 2-hydroxy-6-oxo-(phenyl/chlorophenyl)hexa-2,4-dienoic acid (HOP/cPDA) hydrolase involved in the biphenyl/polychlorinated biphenyl degradation pathway in *Comamonas testosteroni*: evidence suggesting involvement of Ser112 in catalytic activity. *Gene*. 1995 Apr 14;156(1):69-74.

Juteau JM, Billings E, Knox JR, Levesque RC. Site-saturation mutagenesis and three-dimensional modelling of ROB-1 define a substrate binding role of Ser130 in class A beta-lactamases. *Protein Eng*. 1992 Oct;5(7):693-701.

Maclean IW, Slaney L, Juteau JM, Levesque RC, Albritton WL, Ronald AR. Identification of a ROB-1 beta-lactamase in *Haemophilus ducreyi*. *Antimicrob Agents Chemother*. 1992 Feb;36(2):467-9.

Juteau JM, Cote S, Levesque RC. Systematic site-saturation mutagenesis of ROB-1 beta-lactamase: efficiency of T4 polymerase and oligonucleotide synthesis. *Biotechniques*. 1991 Oct;11(4):460-2.

Juteau JM, Sirois M, Medeiros AA, Levesque RC. Molecular distribution of ROB-1 beta-lactamase in *Actinobacillus pleuropneumoniae*. *Antimicrob Agents Chemother*. 1991 Jul;35(7):1397-402.

Juteau JM, Levesque RC. Sequence analysis and evolutionary perspectives of ROB-1 beta-lactamase. *Antimicrob Agents Chemother*. 1990 Jul;34(7):1354-9.

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Mini-review

Respiratory syncytial virus infections: Recent prospects for control

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Dedicated to Prof. Erik De Clercq on the occasion of reaching the status of Emeritus-Professor at the Katholieke Universiteit Leuven in September 2006.

Abstract

Respiratory syncytial virus (RSV) infections remain a significant public health problem throughout the world, although recently developed and clinically approved anti-RSV antibodies administered prophylactically to at-risk populations appear to have significantly affected the disease development. Much effort has been expended to develop effective anti-RSV therapies, using both in vitro assay systems and mouse, cotton rat, and primate models, with several products now in various stages of clinical study. Several products are also being considered for the treatment of clinical symptoms of RSV. In this review, updates on the status of the approved anti-RSV antibodies, ribavirin, and recent results of studies with potential new anti-RSV compounds are summarized and discussed.

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1. Introduction

Respiratory syncytial virus (RSV) infections continue to be a serious public health problem throughout the world. The disease occurs during the winter months in temperate climates and, in the tropics, during the rainy season; evidence is accumulating, however, suggesting the RSV infection may be a year-round event in some areas (Halstead and Jenkins, 1998). The infection is considered the most important cause of lower respiratory tract infections worldwide in infants, particularly those less than 6 months of age, being responsible for high morbidity and mortality (Leung et al., 2005; Anon., 2005). RSV is also a significant cause of respiratory infection among the elderly (Thompson et al., 2003; Falsey and Walsh, 2006) and among bone marrow recipients (Ebbert and Limper, 2005). Vaccines are not available for protection against the RSV infection; indeed, a phenomenon known as “immunopotentialization” or “vaccine-enhanced disease” has been seen in the study of some potential RSV vaccines (Fulginiti et al., 1969; Kapikian et al., 1969; Kim et al., 1969).

The purpose of this review is to consider recent developments in antiviral agents which may have potential for treatment of this important virus infection, as well as to review information about those materials approved for prophylaxis or therapy that are presently in use.

2. In vivo test methodology

Two animal models have routinely been used to evaluate potential RSV inhibitors, these being the mouse and the cotton rat (*Sigmodon hispidus*). Rarely, non-human primates (African green monkeys and chimpanzees) have also been used; the paucity and high cost of primates have discouraged their widespread use, although some laboratories have turned to primate studies for late phases in RSV drug development (see Table 1). The guinea pig can develop an RSV-induced bronchiolitis and manifestations of asthma (Robinson et al., 1996; Bramley et al., 1999, 2003), but has rarely been used for antiviral studies. The mouse and cotton rat models offer similar parameters for evaluation of potential RSV inhibitors: moderate virus titers in tissues of the respiratory tract, and pulmonary histopathology. An effective anti-RSV dose has been defined as a 100-fold reduction in virus load in the lungs (Maggon and Barik, 2004); this is an inhibition generally greater than seen using ribavirin in mice or cotton rats (Table 1). In view of the questionable utility of ribavirin in the clinic (discussed in Section 4.6.1), this $2 \log_{10}$ inhibition standard would appear appropriate, although an even greater titer inhibition would be desirable. Consideration also should be given to histopathological findings, although quantitation of such findings is difficult. Another concern in the interpretation of animal model results is the extrapolation to human outcomes of timing of treatment initiation relative to virus exposure in the animal model. In the laboratory animal, treatment is usually begun within 24 h of virus exposure; however, in the human infant, therapy usually would not begin until perhaps 3 days after manifestations of the RSV disease. Satisfactory comparisons of the

results of animal testing to human studies have not yet been completed.

3. Prophylaxis

3.1. Anti-RSV antibodies

Until a safe and effective antiviral can be developed for treatment of RSV infections, prevention of the infection by use of anti-RSV antibodies appears to be the most acceptable approach. Two antibodies are currently approved for treatment of RSV disease: RSV-IGIV (RespiGam®), which is RSV immune globulin, and palivizumab (Synagis®), which is a chimeric humanized IgG monoclonal antibody, both produced by MedImmune Inc.

The RSV-IGIV is a preparation of polyclonal-concentrated RSV neutralizing antibody obtained from the sera of adult humans. An infusion of 750 mg/kg administered monthly to prematurely born infants has been reported to significantly decrease hospitalization and to reduce the number of hospital days with oxygen (PREVENT Study Group, 1997). However, the product is derived from blood, and consequently, has the potential to transmit blood-borne pathogens; further, its viscosity, coupled with required high volumes for administration, may lead to fluid overload. The material is usually given in a 2–4 h intravenous infusion (Kamal-Bahl et al., 2002). RSV-IGIV must be administered under medical surveillance. With the introduction of palivizumab, use of the product has dramatically declined (Barton et al., 2001).

Palivizumab, a humanized monoclonal antibody directed to an epitope in the A antigenic site of the F-protein of RSV, was approved in 1998 for the prophylaxis of infants at high risk for RSV infection. The product is 50–100 times more potent than RSV-IGIV (Johnson et al., 1997), and is now being used worldwide with considerable success as shown by randomized, double-blind, placebo-controlled trials (Cardenas et al., 2005). Palivizumab is licensed for intramuscular injection of 15 mg/kg administered at monthly intervals throughout the RSV season. No resistance to palivizumab has yet been reported, and all strains of RSV appear to be neutralized by it. Although this product has an excellent record in preterm infants, with a reported 78% exhibiting protection against RSV, infants with bronchopulmonary dysplasia or congenital heart disease have had a significantly lower rate of protection (Cardenas et al., 2005). This lesser effect has been attributed to an insufficient serum concentration, which would be alleviated by using a higher monthly dose (Cardenas et al., 2005).

An improved version of palivizumab, designated as MEDI-524 (Numax™), is now in Phase III clinical evaluation. This antibody appears to bind 70-fold better to the RSV F-protein than palivizumab (Wu et al., 2005). It is more potent in neutralizing RSV in vitro, and in RSV-infected mice (Mejias et al., 2005). Palivizumab exerts its protection by preventing the spread of virus into the lower respiratory tract, thus lessening the clinical manifestations of bronchiolitis; MEDI-524 also inhibits nasal replication of RSV, which may lead to inhibition of upper respiratory tract infections and otitis media (Cardenas et al., 2005).

Table 1
An overview of animal studies with potential RSV-inhibitory compounds

Compound	Animal model	Dosages used	Treatment route	Treatment schedule	Max. tissue virus titer reduction (log ₁₀)	Development status	Reference
Ribavirin	Cotton rat	200 mg/kg	i.p.	tid x 4 beg + 1 h	1.1	Approved 1986; high-risk patients use 1996	Hruska et al. (1982)
	Cotton rat	2 mg/ml	s.d.a.	Continuous beg + 1 h	1.0	Approved 1986; high-risk patients use 1996	Hruska et al. (1982)
	Cotton rat	90 mg/kg/day	i.p.	+24, 48, 72	1.7	Approved 1986; high-risk patients use 1996	Wyde et al. (1990a)
	Cotton rat	60 mg/ml	s.d.a.	2 h bid x 3 beg + 24 h	1.2	Approved 1986; high-risk patients use 1996	Wyde et al. (1987)
VP-14637	Mouse	90 mg/kg/day	i.p.	tid x 3 beg + 24 h	1.8	Approved 1986; high-risk patients use 1996	Sudo et al. (1999)
	Cotton rat	126 µg/kg	s.d.a.	bid x 4 beg + 24 h	2.1	Phase I, discontinued	Wyde et al. (2005)
BMS-433771	Cotton rat	25–200 mg/kg	p.o.	–1 h	~1.0	Preclinical	Cianci et al. (2004a)
	Mouse	5–50 mg/kg	p.o.	bid x 5 beg – 1 h	1.25	Preclinical	Cianci et al. (2004a,c)
RFI-641	Cotton rat	0.2–3.3 mg/kg	i.n.	–2 h	0.6–3.2	Phase I, discontinued	Huntley et al. (2002)
	Mouse	0.08–1.3 mg/kg	i.n.	–2 h	1.5	Phase I, discontinued	Huntley et al. (2002)
	African green monkey	6 mg/kg	i.n.	–2 h	3.4	Phase I, discontinued	Huntley et al. (2002)
	African green monkey	6 mg/kg	i.n.	qd x 8 beg + 24 h	1.6	Phase I, discontinued	Huntley et al. (2002)
JNJ-2408068	Cotton rat	5 mg/ml	s.d.a.	15 min 0 or +24	1.9–3.7	Preclinical	Wyde et al. (2003)
	Cotton rat	5 mg/ml	s.d.a.	+48 or +72 h	0.0	Preclinical	Wyde et al. (2003)
	Cotton rat	5 mg/ml	s.d.a.	–24 h	≥2.0	Preclinical	Wyde et al. (2003)
	Cotton rat	5 mg/ml	s.d.a.	–48 or –96 h	0.0	Preclinical	Wyde et al. (2003)
MBX-300	Cotton rat	100 mg/kg/day	i.p.	qd x 3 beg + 1 h	1.5	Preclinical	Douglas (2004)
SIRNA	Mouse	3.5 µg/kg	i.n.	–4 h, +24 h or +72 h	2.0–3.0	Preclinical	Bitko et al. (2005)
	Mouse	3.5 µg/kg	i.n.	+96 h	<0.5	Preclinical	Bitko et al. (2005)
RBI-034	Cotton rat	50 mg/kg	i.n.	qd x 3 beg + 1 h	1.1	Preclinical	Cramer et al. (2005)
	Cotton rat	50 mg/ml	s.d.a.	qd x 3 beg + 1 h	1.6	Preclinical	Cramer et al. (2005)
	Mouse	10 mg/kg/day	i.n.	–6 h, +24, 72, 120 h	1.3	Preclinical	Cramer et al. (2005)
	African green monkey	50 mg/ml	s.d.a.	–6 h, +24, 72, 120 h	4.0	Preclinical	Leaman et al. (2002)
A-60444	Nr ^a	Nr	Nr	Nr	Nr	Phase II clinical trials	Kelsey et al. (2004), Wilson et al. (2004), Carter et al. (2006)
Compound D	Mouse	0.4–4.1 mg/kg/day	i.n.	+3, 6 h, tid x 3	0.6	Phase II clinical trials	Liuzzi et al. (2005)
VX-497	Nr	Nr	Nr	Nr	Nr	Phase II vs. HBV	Markland et al. (2000)
Mycophenolate mofetil	Mouse	100 mg/kg/day	p.o.	qd x 5 beg + 24 h	Nr	Clinical use as immunosuppressant for transplant rejection	Roberts et al. (2001)
EICAR	Cotton rat	100 mg/kg/day	i.p.	bid x 3 beg + 24 h	1.4	Preclinical	Wyde et al. (2000)
Pyrazofurin	Cotton rat	3 mg/kg/day	i.p.	qd x 3 beg + 24 h	1.0	Preclinical	Wyde et al. (1989)
LY253963	Cotton rat	1 mg/kg/day	i.p.	bid x 3 beg + 24 h	1.3	Phase II vs. influenza, withdrawn	Wyde et al. (1990b)
	Cotton rat	3 mg/kg/day	i.p.	qd x 3 beg + 24 h	1.2	Phase II vs. influenza, withdrawn	Wyde et al. (1990b)
	Cotton rat	10 mg/kg/day	p.o.	qd x 3 beg + 24 h	0.0	Phase II vs. influenza, withdrawn	Wyde et al. (1990b)

^a Not reported.

4. Potential therapeutics

4.1. Virus targets

RSV belongs to the genus *Pneumovirus* of the family Paramyxoviridae. It is an enveloped virus containing a single-stranded, non-segmented, minus sense RNA. The RNA is 15 kDa and encodes 11 proteins, eight of which comprise the virion. Of the eleven proteins, three are surface glycoproteins designated as F-, G-, and the hydrophobic SH-protein. F- and G-proteins protrude through the envelope of the virus and are responsible for attachment (G-protein) and fusion (F-protein) to host cells. The F-protein is cleaved from a precursor F0-protein by cellular enzymes to produce the disulfide-linked F1 and F2 subunits that are the virion F-protein. SH is a small integral membrane protein whose function is not clear. It seems to be phosphorylated by an MAPK p38-dependant tyrosine kinase to achieve its normal cellular distribution in infected cells (Rixon et al., 2005). The inner portion of the envelope interacts with the mature (M) protein, which directs the assembly of virions within the inner side of the host cell membrane from which the viral envelope is derived. Within the envelope, posterior to the M-protein, is the nucleocapsid, which is made up of the major nucleocapsid N-protein that binds to genomic RNA, a phosphoprotein (P), a transcription anti-terminator factor or transcriptase processivity factor (M2-1), and the large polymerase subunit (L), which is a RNA-dependant RNA transcriptase. In addition, there are two non-structural proteins referred to as NS1 and NS2 and a regulatory protein known as M2-2.

Inhibitors of RSV that have been recently evaluated for efficacy fall into five general modes of action groups: those that inhibit attachment/fusion, oligonucleotides that target viral RNA, those that target the N-protein, those that inhibit some function of the virus RNA-dependant RNA polymerase, and those that inhibit inosine monophosphate dehydrogenase (IMPDH), although the latter may or may not represent the actual mode of inhibiting RSV replication.

4.2. Attachment/fusion inhibitors

The F- and G-proteins are involved in virus attachment and fusion, although the F-protein alone is sufficient to promote attachment to cells, subsequently leading to a productive viral infection (Karron et al., 1997). G-protein may simply enhance attachment to a target cell, but F-protein probably binds to a specific receptor (Techarpornkul et al., 2001). The host cell receptor appears to be a glycosaminoglycan containing heparin sulfate (Hallak et al., 2000), since addition of heparin blocks virus attachment in vitro (Krusat and Strockert, 1997). Thus, numerous compounds of various classes have been synthesized which target attachment or the fusion activity of RSV, with fusion inhibitors predominating. The following reviews those that have proceeded to clinical trials and also more recently developed fusion inhibitors not yet announced to be in clinical trials. See Table 1 for an overview of each material which has undergone in vivo study which has been discussed in this review.

4.2.1. VP-14637

VP-14637 (5,5'-bis[1-(((5-amino-1*H*-tetrazolyl)imino)-methyl)] 2,2',4''-methylidynetrisphenol; Fig. 1) was one of the first fusion inhibitors to progress to Phase I clinical trials. It is a triphenolic compound which apparently binds into the small hydrophobic cavity in the inner core of the F-protein, either preventing early transient Z conformation changes in the fusion process or by preventing formation of six-helix fusion core as the heptad repeats interact (Douglas et al., 2005). VP-14637 is a broad-spectrum inhibitor of RSV strains, inhibiting the virus in vitro at concentrations of 2 nM or less (Douglas et al., 2005; Wyde et al., 2005). In cotton rats, treatment by small droplet aerosol for 60 min significantly reduced mean lung virus titers (Wyde et al., 2005). VP-14637 was in phase I trials prior to a decision not to develop it further, in part due to developmental costs.

4.2.2. BMS-433771

Benzotriazole benzimidazoles represent another class of inhibitors that prevent fusion of RSV to host cell membranes. In particular, BMS-433771 (1-cyclopropyl-1,3-dihydro-3-[[1-(3-hydroxypropyl)-1*H*-benzimidazol-2-yl]methyl]-2*H*-imidazo[4,5-*c*]pyridin-2-one; Fig. 1), an azabenzimidazolone derivative, targets the hydrophobic pocket within the trimer of hairpins of the F-1 protein, a class I fusion protein (Cianci et al., 2004b). It apparently interferes with the normal association of the N- and C-terminal heptad repeats found within the binding pocket that occur as part of the fusion process (Cianci et al., 2005). BMS-433771 is active against multiple RSV strains, with a 50% inhibitory concentration (IC₅₀) of 20 nM (Cianci et al., 2004b). In a T cell-deficient BALB/c mouse model, the orally active compound (50 µg/kg) also significantly reduced virus titers in the lungs. The compound was well tolerated. The EC₅₀ in the BALB/c mouse was determined to be 12 nM. The compound was somewhat less inhibitory to lung virus replication in cotton rats than in mice (Cianci et al., 2004a,c).

4.2.3. RFI-641

RFI-641 (4,4'-bis-4,6-bis-[3-(bis-carbamoylmethyl-sulfamoyl)-phenylamino]-[1,3,5] triazin-2-ylamino-biphenyl-2,2'-disulfonic acid; Fig. 1) from Wyeth-Ayerst (Pearl River, NY) is biphenyl triazine anionic compound that is an analog of CL-309623, a previously identified dendrimer-like stilbene inhibitor with anti-RSV activity (Gazumyan et al., 2000). Like the parent compound, RFI-641 inhibits RSV fusion mediated by the F-protein by directly interacting with that protein (Razinkov et al., 2002). The compound inhibited both A and B strains of RSV with EC₅₀ values in the 20 nM range. It was also relatively non-toxic with selective indices ranging from 417 to 2500 (Douglas, 2004). The drug has been evaluated extensively in small animal models (Huntley et al., 2002) and in African green monkeys (Weiss et al., 2003). In the mouse, RFI-641 at 1.3 mg/kg delivered intranasally 2 h prior virus exposure reduced virus lung titers by 1.5 log₁₀ plaque-forming units. In cotton rats using a similar prophylactic dosing regimen, doses up to 10 mg/kg reduced lung virus titers by >3 log₁₀. In the primate, RFI-641 prophylactically dosed at 6 mg reduced

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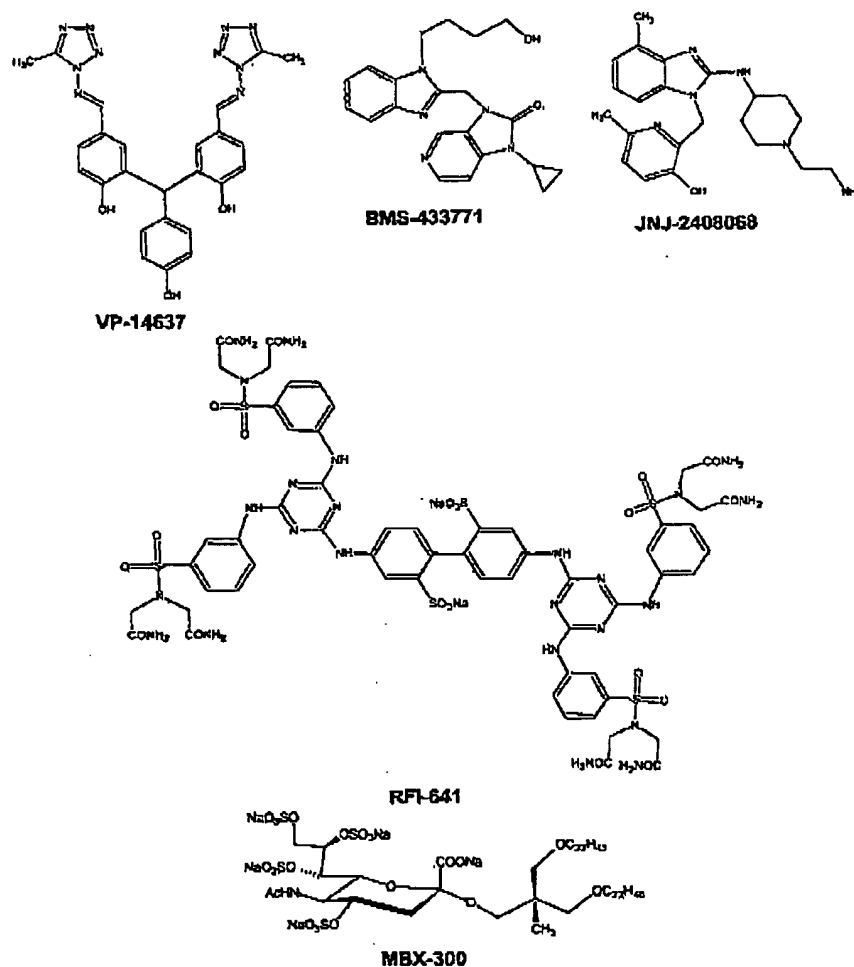


Fig. 1. Fusion inhibitors.

nasal virus titers by $>3.4 \log_{10}$ over a period of 10 days. Using intranasal administration, lung virus titers were only substantially reduced after a 2 h exposure to the compound (Weiss et al., 2003). When given 24 h after virus exposure and using daily doses thereafter, nasal virus titers were also significantly reduced (Huntley et al., 2002).

RFI-641 was in Phase II clinical trials in 2000–2001 for the secondary prevention and therapeutic treatment of RSV infections in adults (<http://www.uchsc.edu/peds/research/rf/cio/rcsfund/index.htm>).

4.2.4. JNJ-2408068

JNJ-2408068 (2-[[[1-(2-aminoethyl)-4-piperidinyl]amino]-4-methyl-1H-benzimidazol-1-yl]-6-methyl-3-pyridinol]; Fig. 1), is being developed by Johnson & Johnson (Raritan, NJ). It has very similar mode of inhibition to VP-14637 discussed earlier (Douglas et al., 2005). The compound is potent in vitro ($EC_{50} = 0.16 \text{ nM}$), is not cytotoxic at concentrations $>100 \text{ } \mu\text{M}$, and inhibits all members of the pneumovirus genera except for the murine pneumonia virus. In cotton rats, the compound was

administered by aerosol for 15 min, either prior to or after virus exposure. Lung virus titers were reduced to below detectable limits (Wyde et al., 2003). It appeared well tolerated, but has limited oral bioavailability (Douglas, 2004).

4.2.5. MBX 300 (NMSO-3)

MBX 300 (Microbiotix, Worcester, MA) is [2,2-bis(docosyl-oxymethyl)propyl-5-acetoamido-3,5-dideoxy-4,7,8,9-tetra-O-(sodium-oxysulfonyl)-D-glycero-D-galacto-2-nonulopyranosid]onate (Fig. 1). The compound apparently targets the attachment phase because its target is the G-protein. It is a specific inhibitor of RSV (Kimura et al., 2000) with EC_{50} values from 0.2 to $0.3 \text{ } \mu\text{M}$ (Douglas, 2004). In cotton rats, when administered intraperitoneally at 100 mg/kg/day , lung virus titers were significantly reduced 3 days post-virus exposure (Douglas, 2004). According to Microbiotix, MBX 300 has undergone preliminary toxicology studies, including testing in cynomolgus monkeys (<http://www.microbiotix.com/pr030502.htm>) and has specific and potent oral anti-RSV activity as well as an excellent safety profile. Microbiotix will be advancing MBX

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300 through preclinical development and expects to initiate clinical studies in the near future.

4.2.6. Small peptide fusion inhibitors

Recently, three peptides containing multiple copies of alternating HR1 and HR2 sequences of the terminal heptad repeats of the F-protein and denoted as 5-helix, HR121 and HR212 were designed to inhibit F-protein mediated fusion (Ni et al., 2005). The 5-helix, HR121 and HR212 proteins were functionally analogous to single HR1, HR1, and HR2 sequences of terminal ends of the F-protein, respectively. The three proteins were potent fusion inhibitors in vitro with IC_{50} values ranging from 3 to 8 μ M as determined by a visual syncytial reduction assay. These peptides represent a targeted design approach for discovery of fusion inhibitors and could be lead compounds for the development of peptide RSV-peptide inhibitors.

4.3. Oligonucleotides that target viral RNA (antisense/siRNA)

Much has been reviewed regarding the theory, approaches used, and attempts to develop antisense oligonucleotides as therapies for RSV disease (Maggon and Barik, 2004; Cramer, 2005; Leaman, 2005). Strategies pursued have included antisense phosphorothioate oligodeoxyribonucleotides (ODN) (Jairath et al., 1997), short interfering double-stranded RNA molecules (siRNA) (Bitko et al., 2005), and 2–5 Å antisense chimeras (Barnard et al., 1999; Torrence, 1999). One phosphorothioate ODN targeted to repetitive intergenic sites with the RSV genome appeared significantly effective versus the virus (Jairath et al., 1997), but development never proceeded into the clinic, due, in part, to side effects (Siddiqui-Tain et al., 2002). Much study is underway with the siRNAs; a recent report by Bitko et al. (2005) showed that intranasal instillation of an in vitro-active siRNA into RSV-infected mice was significantly inhibitory to the infection. Treatment begun 4 h before the virus infection reduced the lung virus titers by 3 log₁₀ and prevented pulmonary pathology from developing. When therapy began after virus exposure, the antiviral effect was progressively less, but continued to lower the virus titers. It should be noted that an siRNA being developed by Alnylam Pharmaceuticals, designated as ALN-RSV01, is claimed to be significantly inhibitory to RSV in vitro and in animal models, and the product, to be administered directly to the lungs, is now in Phase I clinical trials (Thomson CenterWatch Clinical Trials Listings Service; <http://www.centerwatch.com/professional/cwpipeline/>). Nothing has yet been published regarding the antiviral activity of ALN-RSV01. The use of 2–5 Å antisense strategy is also in developmental stages, but data have been recently published indicating this approach may also have promise. The 2–5 Å antisense chimera designed RBI034 has demonstrated potent anti-RSV efficacy in vitro (Xu et al., 2004) and aerosolized delivery reduced RSV infections in mice, cotton rats, and African green monkeys (Leaman et al., 2002; Cramer, 2005). Of potential significance is the observation that the combination therapy using RBI034 with ribavirin was more effective than either material used alone (Cramer et al., 2005). Some technical problems

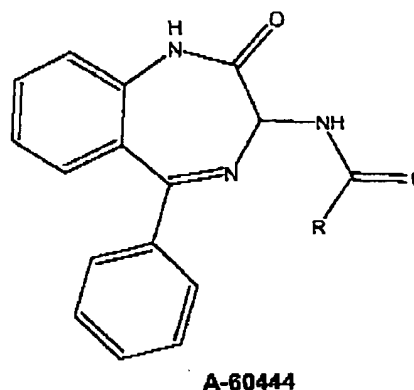


Fig. 2. N-protein inhibitor.

have slowed the progress of antisense antivirals; these include enhanced delivery to the target cells, a need to improve stability, a wider therapeutic window, and the challenge of large-scale synthesis (Maggon and Barik, 2004; Leaman, 2005).

4.4. N-protein inhibitors

A-60444 (RSV-604) is a 1,4-benzodiazepine derivative with the general structural configuration as shown in Fig. 2 (Kelsey et al., 2004; Carter et al., 2006). In resistant mutant studies, the compound was found to be unique in that it apparently targets the N-protein of RSV (Wilson, 2004). The in vitro inhibitory activity is in the submicromolar range for both A and B RSV (Wilson et al., 2004). In Phase I clinical trials in the United Kingdom the compound was found safe and well tolerated without any serious adverse effects (Thomson CenterWatch Clinical Trials Listings Service; <http://www.centerwatch.com/professional/cwpipeline/>).

Pharmacokinetic studies from this trial suggested that once daily dosing was feasible. The compound has now entered Phase II clinical trials (<http://www.clinicaltrials.gov/ct/show/NCT00232635?order=1>) to evaluate the antiviral effect of nasal/oral administration versus placebo in post-stem cell transplant patients with RSV infection and to assess the safety of the product. The pharmacokinetics of A-60444 in the presence of concomitant medications such as immunosuppressants and anti-fungal agents will also be studied. The open-label portion of the trial is now complete and the placebo-controlled trials are underway (Powell, CEO, Arrow Pharmaceuticals, personal communication). It is disappointing that detailed in vivo anti-RSV data are not yet available for this compound so that comparisons can be made to the other potential RSV inhibitors described in this review.

4.5. RNA-dependant RNA polymerase inhibitors

A number of imidazo[4,5-h]isoquinoline-7,9-dione inhibitors were synthesized that targeted the guanylation of viral transcripts (5' cap) of the RSV ribonucleoprotein (RNP) complex (Liu et al., 2005). The most potent of these inhibitors was

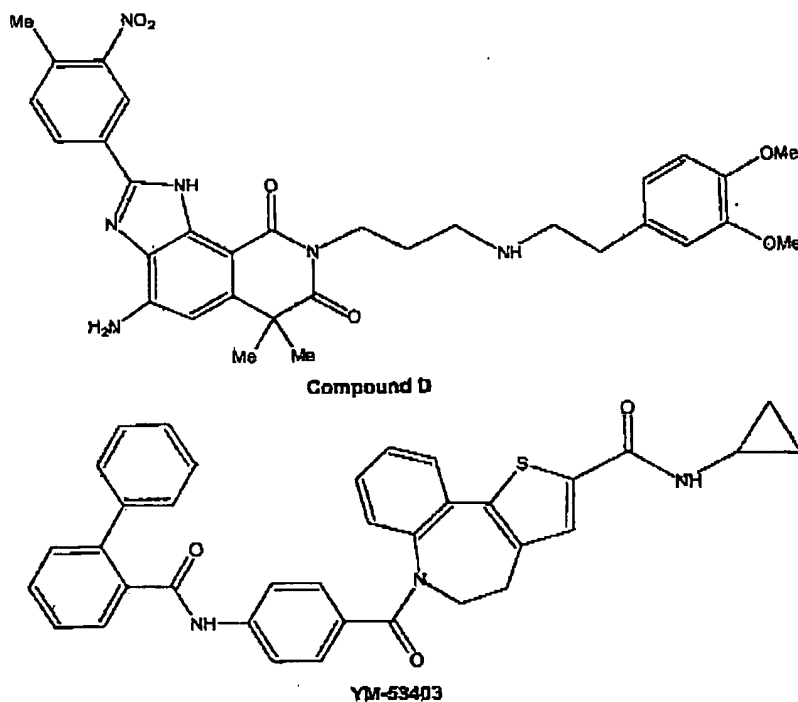


Fig. 3. RNA-dependent RNA polymerase inhibitors.

4-amino-8-(3-{[2-(3,4-dimethoxyphenyl)ethyl]amino}propyl)-6,6-dimethyl-2-(4-methyl-3-nitrophenyl)-1*H*-imidazo[4,5-*h*]-isoquinoline-7,9(6*H*,8*H*)-dione (Compound D; Fig. 3). These inhibitors may bind to a region in the L-protein with similarities to NDK motifs; NDK proteins play a role in maintaining the balance of intracellular nucleotide pools by exchanging gamma-phosphate groups from NTP to NDP. These compounds inhibited RSV replication in an ELISA-based assay with EC₅₀ values ranging from 0.021 to 2.1 μ M. Selectivity indices ranged from 30 to 400. In a mouse model, lung virus titers were reduced when the compounds were administered intranasally 3 and 6 h after virus exposure, then three times daily for 3 days at 0.4–4.1 mg/kg/day.

Recently, a novel benzazepine inhibitor of the L-protein was discovered from a large chemical library; it was designated as YM-53403 (6-{4-[(biphenyl-2-yl)carbonyl]amino}benzoyl)-*N*-cyclopropyl-5,6-dihydro-4*H*-thieno[3,2-*d*][1]benzazepine-2-carboxamide (Fig. 3) (Sudo et al., 2005). In a plaque reduction assay, the compound inhibited RSV replication at 0.2 μ M. Mutant viruses with single point mutations in the L-protein (virus polymerase) were resistant to the antiviral effects of the compound and timing studies suggested that inhibition was maximal at around 8 h after virus exposure.

4.6. Inosine monophosphate dehydrogenase inhibitors

4.6.1. Ribavirin

Ribavirin (1- β -D-ribofuranosyl-1,2,4-triazole-3-carboxamide, Virazole[®]) is the only antiviral drug currently

approved for treatment of RSV infections. Ribavirin has inhibitory effects on a very broad spectrum of viruses, including RSV (Sidwell et al., 1972). The mechanism of viral inhibition by the drug is best described as multi-faceted and includes inhibition of IMPDH, inhibition of the 5' cap formation of mRNA, and inhibition of viral polymerase by the phosphorylated forms of the compound, although the specific mechanism by which RSV is inhibited is not well documented (Sidwell, 1996). In early clinical studies, significant positive effects in RSV-infected infants were reported using ribavirin administered in a small-particle aerosol (Hall et al., 1983a,b; Taber et al., 1983; McIntosh et al., 1984; Barry et al., 1986); however, water was used as the placebo and has a bronchoconstricting effect by itself which may have affected the outcome of the studies. Subsequent trials, using saline as placebo, did not demonstrate the positive effects initially observed (Broughton and Greenough, 2004). Later studies have suggested that the aerosolized ribavirin may lessen post-bronchiolitic wheezing and reactive airway disease and reduce the viral load in the patient, but does not reduce the duration of hospitalization (Edell et al., 1999, 2002; Rodriguez et al., 1999; Guerguerian et al., 1999). Side effects (possible deterioration of respiratory function, anemia, teratogenicity) may also discourage use of this drug.

4.6.2. Other IMPDH inhibitors

The enzyme IMPDH catalyzes the conversion of inosine monophosphate to xanthosine monophosphate, which is an essential step in the de novo biosynthesis of guanine nucleotides

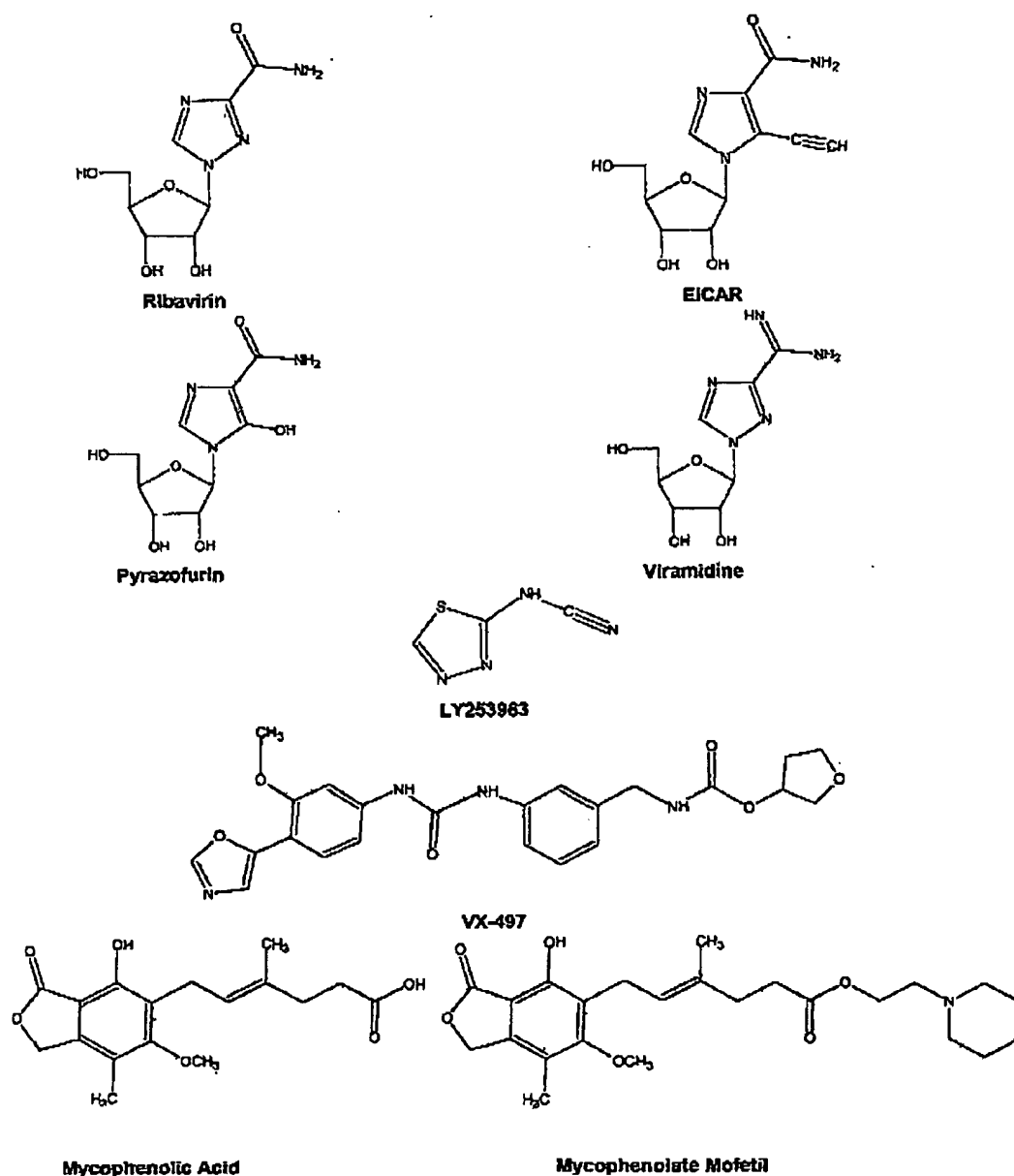


Fig. 4. IMPDH inhibitors.

leading to DNA and RNA synthesis. Inhibition of IMPDH thus reduces the amount of intracellular guanine nucleotides needed for RNA and DNA synthesis and consequently can result in significant antiviral effects, although such effects may also be associated with inhibition of cell replication. A number of compounds in addition to ribavirin, which are considered IMPDH inhibitors, have exhibited significant anti-RSV activity. These include VX-497, mycophenolic acid, mycophenolate mofetil, EICAR, pyrazomycin, viramidine, and LY253963 (Fig. 4).

VX-497 is a selective, highly potent, reversible, and uncompetitive inhibitor of IMPDH; it is structurally unrelated to other

IMPDH inhibitors. The compound has a broad-spectrum antiviral effect, inhibiting RSV with a 50% inhibitory concentration (IC_{50}) of 1.1 μ M and a 50% cytotoxic concentration (CC_{50}) of 10.2 μ M (Markland et al., 2000). This activity was approximately 20-fold more potent than ribavirin, but the therapeutic index of VX-497 was less than that of ribavirin. The compound, in combination with interferon alpha, is currently being developed by Vertex Pharmaceuticals (Cambridge, MA) for the treatment of hepatitis C (McHutchison et al., 2005).

Mycophenolic acid has a broad-spectrum antiviral effect similar to that of ribavirin (Ando et al., 1968; Cline et al., 1969;

Planterose, 1969), although RSV was not included among the viruses initially studied. Roberts et al. (2001) have described the compound to be more potent than ribavirin both as an antiviral agent against RSV and as an inhibitor of IMPDH. In vitro studies we have done (unpublished data) indicate the IC_{50} of mycophenolic acid versus the A2 strain of RSV was 0.2–0.7 $\mu\text{g/ml}$ compared to ribavirin's IC_{50} of 1–4 $\mu\text{g/ml}$, but the compound was also more cytotoxic, the CC_{50} being 10 $\mu\text{g/ml}$ compared to a value of 80–120 $\mu\text{g/ml}$ for ribavirin. Mycophenolic acid is a recognized immunosuppressant (Mitsui and Suzuki, 1969). Mycophenolate mofetil (CellCept), the prodrug of mycophenolic acid, is used as an immunosuppressive in the therapy of transplant rejection (Danovitch, 2005). A single report (Roberts et al., 2001) has indicated oral administration of the compound significantly inhibited RSV-induced pneumonia in mice, with efficacy still seen when treatment initiation was delayed to 5 days after virus exposure.

EICAR (5-ethynyl-1-beta-D-ribofuranosylimidazole-4-carboxamide) was initially reported by De Clercq et al. (1991) to have significant in vitro activity versus RSV, the IC_{50} being 0.2 $\mu\text{g/ml}$. Cytotoxicity was not seen at 400 $\mu\text{g/ml}$, the highest concentration evaluated. Significant efficacy was also seen using i.p. treatment of RSV-infected cotton rats (Wyde et al., 2000).

Pyrazofurin is another IMPDH inhibitor with significant RSV inhibitory effects, the IC_{50} ranging from 0.02 to 1 $\mu\text{g/ml}$, depending upon the virus strain (Kawana et al., 1985, 1987). Efficacy was also seen versus RSV in the cotton rat model treated with this compound (Wyde et al., 1989).

Viramidine (ribamidine), the 3-carboxamidine analog of ribavirin being developed by Valeant Pharmaceuticals (Costa Mesa, CA), has an anti-RSV IC_{50} of 16 $\mu\text{g/ml}$, which is slightly higher than that of ribavirin, but is also less cytotoxic, with a CC_{50} of >1000 $\mu\text{g/ml}$ (Gabrielsen et al., 1992). No animal studies with RSV have been reported; however, studies we have run (Sidwell et al., 2005) with viramidine in comparison to ribavirin versus influenza virus infections in mice suggest that both compounds have a similar therapeutic index, although viramidine is not taken up by red blood cells in the efficient manner that is seen with ribavirin and hence appears to have an enhanced safety profile (Lin et al., 2003).

LY253963, the sodium salt of 1,3,4-thiadiazole-2-ylcyanamide and the prodrug of an IMPDH inhibitor was reported by Wyde et al. (1990b) to have in vitro efficacy against RSV that was approximately equivalent to that of ribavirin; intraperitoneal treatment of RSV-infected cotton rats was also protective, but oral therapy was not effective in the same study. The compound, also known to be a significant inhibitor of influenza virus (Hayden et al., 1990), failed in a clinical trial against influenza (Hayden et al., 1994) and has been reported to have developmental toxicity effects (Herman and Chay, 1998). In these latter studies, it was designated as LY217896.

The pyrazole dicarboxamide analog of ribavirin, designated as GR92938X, has been reported to be an inhibitor of RSV in vitro without inhibiting other viruses such as influenza and parainfluenza; it may be pertinent to note that this compound did not appear to inhibit IMPDH (Woods et al., 1994).

5. Treating RSV bronchiolitis

Finally, a number of compounds have been or are now in clinical trials to treat bronchiolitis, the inflammatory disease caused by RSV infection, as well as the reactive airway disease developing after the bronchiolitis (Bisgaard, 2003). Clinical trials with anti-inflammatories have often failed to demonstrate significant effects. Systemically administered prednisolone (Bulow et al., 1999) and topically applied fluticasone (Wong et al., 2000), budesonide (Cade et al., 2000), and deoxyribonuclease I (Nasr et al., 2001) were not found useful. Dexamethazone given by intravenous injection (Buckingham et al., 2002) also had little effect, although when administered by inhalation was concluded to possibly reduce length of hospitalization (Bentur et al., 2005). Porcine surfactant administered intratracheally to RSV-infected infants was considered to have some beneficial effect as seen by improved gas exchange and lessened conventional mechanical ventilation (Luchetti et al., 2002). Most of these therapies target the hyper-inflammatory response and not the agent inducing this response. A fully effective treatment might need to be a combination of anti-inflammatory agents to alleviate the life-threatening symptoms and a potent antiviral agent to eliminate the source of the inflammatory response. It is notable that a combination of palivizumab and a glucocorticosteroid had a significant effect in lessening pulmonary histopathology and also markedly reduced lung virus titers over 3 log₁₀ in RSV-infected cotton rats (Prince et al., 2000). Such a combination would be especially valuable in patients who are not immunocompetent or are immunologically immature.

6. Conclusions

At present, the most effective means for control of RSV infections is the use of anti-RSV antibodies (RSV-IGIV, palivizumab, MEDI-524) administered prophylactically to at-risk patients. The RSV-IGIV has essentially been replaced now by the other, more recent and improved antibodies. Ribavirin, approved for clinical RSV use, has lost favor as a therapy for RSV infections. Selected compounds which have shown great promise in vitro and in animal models and are now in some phase of clinical study, include the fusion inhibitors RFI-641 and MBX 300, one or more antisense siRNA's, and the N-protein inhibitor A-60444. Anti-inflammatories including dexamethazone and porcine surfactant have exhibited some promise in clinical trials to reduce symptomatology associated with RSV disease; use of these materials in combination with a drug which has specific anti-RSV activity may be an effective approach for better control of this disease.

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References

- Ando, K., Suzuki, S., Tamura, G., Arima, K., 1968. Antiviral activity of mycophenolic acid studies on antiviral and antitumor antibiotics. Part IV. *J. Antibiot.* 21, 649–652.
- Anon, 2005. Brief report: respiratory syncytial virus activity — United States, 2004–2005. *MMWR Morb. Mortal. Wkly. Rep.* 54, 1259–1260.
- Barnard, D.L., Sidwell, R.W., Xiao, W., Player, M.R., Adah, S.A., Torrence, P.F., 1999. 2–5 Å-DNA conjugate inhibition of respiratory syncytial virus replication: effects of oligonucleotide structure modifications and RNA target site selection. *Antiviral Res.* 41, 119–134.
- Barry, W., Cockburn, F., Cronall, R., Price, J.F., Sutherland, G., Vardag, A., 1986. Ribavirin aerosol for acute bronchiolitis. *Arch. Dis. Child.* 61, 593–597.
- Barton, L.L., Grant, K.L., Lemen, R.J., 2001. Respiratory syncytial virus immune globulin: decisions and costs. *Pediatr. Pulmonol.* 32, 20–28.
- Bentur, L., Shoseyov, D., Feigenbaum, D., Gorichovsky, Y., Bibi, H., 2005. Dexamethasone inhalations in RSV bronchiolitis: a double-blind, placebo-controlled study. *Acta Paediatr.* 94, 866–871.
- Bisgaard, H., 2003. A randomized trial of montelukast in respiratory syncytial virus postbronchiolitis. *Am. J. Respir. Crit. Care Med.* 167, 379–383.
- Bifko, V., Maslyenko, A., Shulyayeva, O., Aarik, S., 2005. Inhibition of respiratory viruses by nasally administered siRNA. *Nat. Med.* 11, 50–55.
- Bramley, A.M., Vialls, T.Z., Wiggs, B.R., Hegele, R.G., 1999. Effects of respiratory syncytial virus persistence on airway responsiveness and inflammation in guinea pigs. *Eur. Respir. J.* 14, 1061–1067.
- Bramley, A.M., Khan, M.A., Manson, H.E., Hegele, R.G., 2003. Development of respiratory syncytial virus “bronchiolitis” in guinea pigs does not reflect an allergic predisposition in the host. *Chest* 124, 671–681.
- Broughton, S., Greenough, A., 2004. Drugs for the management of respiratory syncytial virus infection. *Curr. Opin. Invest. Drugs* 5, 862–865.
- Buckingham, S.C., Jaffri, H.S., Bush, A.J., Carubelli, C.M., Sheeran, P., Hardy, R.D., Ottoline, M.G., Ramilo, O., DeVincenzo, J.P., 2002. A randomized, double-blind, placebo-controlled trial of dexamethasone in severe respiratory syncytial virus (RSV) infection: effects on RSV quantity and clinical outcome. *J. Infect. Dis.* 185, 1222–1228.
- Bulow, S.L., Nir, M., Levin, E., Frits, B., Thomsen, L.L., Nielsen, J.E., Holm, J.C., Møller, T., Bonde-Hansen, M.E., Nielsen, H.E., 1999. Prednisolone treatment of respiratory syncytial virus infection: a randomized controlled trial of 147 infants. *Pediatrics* 104, 77–82.
- Cade, A., Brownlee, K.G., Cunney, S.P., Haigh, D., Short, A., Brown, J., Dassu, D., Mason, S.A., Phillips, A., Eglin, R., Graham, M., Chetcuti, A., Chatrathi, M., Hudson, N., Thomas, A., Chetcuti, P.A., 2000. Randomised placebo controlled trial of nebulised corticosteroids in acute respiratory syncytial virus bronchitis. *Arch. Dis. Child.* 82, 126–130.
- Cardenas, S., Ansis, A., Piedimonte, G., 2005. Palivizumab in the prophylaxis of respiratory syncytial virus infection. *Expert Rev. Anti-infect. Ther.* 3, 719–726.
- Carter, M.C., Alber, D.G., Baxter, R.C., Bithell, S.K., Budworth, J., Chubb, J., Dowdell, V.C.L., Henderson, E.A., Keegan, S.J., Kelsey, R.D., Lockyer, M.J., Stables, J.N., Wilson, L.J., Powell, K.L., 2006. 1,4-Benzodiazepines as inhibitors of respiratory syncytial virus. *J. Med. Chem.* 49, 2311–2319.
- Cianci, C., Yu, K.L., Cornbrink, K., Sin, N., Pearce, B., Wang, A., Civitello, R., Voss, S., Luo, G., Kadow, K., Genovesi, E.V., Venables, B., Gulgeze, H., Trohan, A., James, J., Lamb, L., Medina, I., Roach, J., Yang, Z., Zadjura, L., Colonna, R., Clark, J., Meanwell, N., Krystal, M., 2004a. Orally active fusion inhibitor of respiratory syncytial virus. *Antimicrob. Agents Chemother.* 48, 413–422.
- Cianci, C., Langley, D.R., Dischino, D.D., Sun, Y., Yu, K.-L., Stanley, A., Roach, J., Li, Z., Dallacchio, R., Colonna, R., Meanwell, N.A., Krystal, M., 2004b. Targeting a binding pocket within the trimer-of-hairpins: small-molecule inhibition of viral fusion. *PNAS* 101, 15046–15051.
- Cianci, C., Genovesi, E.V., Lamb, L., Medina, I., Yang, Z., Zadjura, L., Yang, H., D’Arienzo, C., Sin, N., Yu, K.-L., Cornbrink, K., Li, Z., Colonna, R., Meanwell, N., Clark, J., Krystal, M., 2004c. Oral efficacy of a respiratory syncytial virus inhibitor in rodent models of infection. *Antimicrob. Agents Chemother.* 48, 2448–2454.
- Cianci, C., Meanwell, N., Krystal, M., 2005. Antiviral activity and molecular mechanism of an orally active respiratory syncytial virus fusion inhibitor. *J. Antimicrob. Chemother.* 55, 289–292.
- Cline, J.C., Nelson, J.D., Gerzon, K., Williams, R.H., DeLong, D.C., 1969. In vitro antiviral activity of mycophenolic acid and its reversal by guanine-type compounds. *Appl. Microbiol.* 18, 14–20.
- Cramer, H., 2005. Antisense approaches for inhibiting respiratory syncytial virus. *Expert Opin. Biol. Ther.* 5, 207–220.
- Cramer, H., Okicki, J.R., Kuang, M., Xu, Z., 2005. Targeted therapy of respiratory syncytial virus by 2–5 Å antisense. *Nucleos. Nucleot. Nucleic Acids* 24, 497–501.
- Damovitch, G.M., 2005. Mycophenolate mofetil: a decade of clinical experience. *Transplantation* 80 (Suppl. 2), S272–S274.
- De Clercq, E., Coels, M., Balzarini, J., Snoeck, R., Andrei, G., Hosoya, M., Shigeta, S., Ueda, T., Minakawa, N., Matsuda, A., 1991. Antiviral activities of 5-ethynyl-1-β-D-ribofuranosylimidazole-4-carboxamide and related compounds. *Antimicrob. Agents Chemother.* 35, 679–684.
- Douglas, J.L., 2004. In search of a small-molecule inhibitor for respiratory syncytial virus. *Expert Rev. Anti-infect. Ther.* 2, 625–639.
- Douglas, J.L., Panis, M.L., Ho, E., Lin, K.-Y., Krawczyk, S.H., Grant, D.M., Cai, R., Swaminathan, S., Chen, X., Cihlar, T., 2005. Small molecules VP-14637 and JNJ-2408068 inhibit respiratory syncytial virus fusion by similar mechanisms. *Antimicrob. Agents Chemother.* 49, 2460–2466.
- Ehbert, J.O., Limper, A.H., 2005. Respiratory syncytial virus pneumonia in immunocompromised adults: clinical features and outcome. *Respiration* 72, 263–269.
- Edell, O., Bruce, E., Hale, K., Edell, D., Khosheer, V., 1999. Reduced long-term respiratory morbidity after treatment of respiratory syncytial virus bronchiolitis with ribavirin in previously healthy infants. A preliminary report. *Pediatr. Pulmonol.* 25, 154–158.
- Edell, O., Khosheer, V., Ross, G., Salter, K., 2002. Early ribavirin treatment of bronchiolitis: effect on long-term respiratory morbidity. *Chest* 122, 935–939.
- Falsey, A.R., Walsh, E.E., 2006. Viral pneumonia in older adults. *Clin. Infect. Dis.* 15, 518–524.
- Fulginiti, V.A., Eller, J.J., Sieber, O.F., Joyner, J.W., Minamitani, M., Meiklejohn, G., 1969. Respiratory virus immunization. Part I. A field trial of two inactivated respiratory virus vaccines; an aqueous trivalent parainfluenza virus vaccine and an alum-precipitated respiratory syncytial virus vaccine. *Am. J. Epidemiol.* 89, 435–448.
- Gabrielsen, B., Phelan, M.J., Barthel-Rosa, L., See, C., Huggins, J.W., Kefauver, D.F., Monath, T.P., Ussery, M.A., Churny, G.N., Schubert, E.M., 1992. Synthesis and antiviral evaluation of *N*-carboxamide-substituted analogues of 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide hydrochloride. *J. Med. Chem.* 35, 3231–3238.
- Gazumyan, A., Mitsner, B., Ellestad, G.A., 2000. Novel anti-RSV dimeric dendrimer-like compounds: design, synthesis and biological evaluation. *Curr. Pharm. Des.* 6, 525–546.
- Guerguerian, A.M., Gauthier, M., Lebel, M.H., Farrell, C.A., Lacroix, J., 1999. Ribavirin in ventilated respiratory syncytial virus bronchiolitis: a randomized, placebo-controlled trial. *Am. J. Respir. Crit. Care Med.* 160, 829–834.
- Hall, C.B., McBride, J.T., Walsh, E.E., Bell, D.M., Gala, C.L., Hildreth, S., Ten Eyck, L.G., Hall, W.J., 1983a. Aerosolized ribavirin treatment of infants with respiratory syncytial viral infection. A randomized double-blind study. *N. Engl. J. Med.* 308, 1443–1447.
- Hall, C.B., Walsh, E.E., Hruska, J.F., Betts, R.F., Hall, W.J., 1983b. Ribavirin treatment of experimental respiratory syncytial viral infection. A controlled double-blind study in young adults. *JAMA* 249, 2666–2670.
- Hallak, L.K., Spillmann, D., Collins, P.L., Peeples, M.E., 2000. Glycosaminoglycan sulfation requirements for respiratory syncytial virus infection. *J. Virol.* 74, 10508–10513.
- Halstead, D.C., Jenkins, S.G., 1998. Continuous non-seasonal epidemic of respiratory syncytial virus infection in the southeast United States. *S. Med. J.* 91, 433–436.
- Hayden, F.G., Rollins, B.S., Hay, A.J., 1990. Anti-influenza virus activity of the compound LY253963. *Antiviral Res.* 14, 25–38.

- Hayden, F.G., Tunkel, A.R., Treanor, J.J., Betts, R.F., Allerheiligen, S., Harris, J., 1994. Oral LY27896 for prevention of experimental influenza A virus infection and illness in humans. *Antimicrob. Agents Chemother.* 38, 1178–1181.
- Herman, J.L., Chay, S.H., 1998. Quantitative whole-body autoradiography in pregnant rabbits to determine fetal exposure of potential teratogenic compounds. *J. Pharmacol. Toxicol. Meth.* 39, 29–33.
- Hruska, J.F., Morrow, P.E., Sufin, S.C., Douglas Jr., R.G., 1982. In vivo inhibition of respiratory syncytial virus by ribavirin. *Antimicrob. Agents Chemother.* 21, 125–130.
- Huntley, C.C., Weiss, W.J., Gazumyan, A., Buklan, A., Feld, B., Hu, W., Jones, T.R., Murphy, T., Nikitenko, A.A., O'Hara, B., Prince, G., Quartuccio, S., Raifeld, Y.E., Wyde, P., O'Connell, J.F., 2002. RFI-641, a potent respiratory syncytial virus inhibitor. *Antimicrob. Agents Chemother.* 46, 841–847.
- Jairath, S., Vargas, P.B., Hamlin, H.A., Field, A.K., Kilkuskie, R.E., 1997. Inhibition of respiratory syncytial virus replication by antisense oligodeoxynucleotides. *Antiviral Res.* 33, 201–213.
- Johnson, S., Oliver, C., Prince, G.A., Hemming, V.G., Pfaff, D.S., Wang, S.C., Dormitzer, M., O'Grady, J., Koenig, S., Tamura, J.K., Woods, R., Bansal, G., Couchenour, D., Tsao, E., Hall, W.C., Young, J.F., 1997. Development of a humanized monoclonal antibody (MEDI-493) with potent in vitro and in vivo activity against respiratory syncytial virus. *J. Infect. Dis.* 176, 1215–1224.
- Kamal-Bahl, S., Doshi, J., Campbell, J., 2002. Economic analyses of respiratory syncytial virus immunoprophylaxis in high-risk infants — a systematic review. *Arch. Pediatr. Adolesc. Med.* 256, 1034–1041.
- Kapikian, A.Z., Mitchell, R.H., Chanock, R.M., Shvedoff, R.A., Stewart, C.E., 1969. An epidemiologic study of altered clinical reactivity to respiratory syncytial (RS) virus infection in children previously vaccinated with an inactivated RS vaccine. *Am. J. Epidemiol.* 89, 405–421.
- Karron, R.A., Buonagurio, D.A., Georgiu, A.F., Whitehead, S.S., Adamus, J.E., Clements-Mann, M.L., Harris, D.O., Randolph, V.B., Udem, S.A., Murphy, B.R., Sidhu, M.S., 1997. Respiratory syncytial virus (RSV) SH and G proteins are not essential for viral replication in vitro: clinical evaluation and molecular characterization of a cold-passaged, attenuated RSV subgroup B mutant. *Proc. Natl. Acad. Sci. U.S.A.* 94, 13961–13966.
- Kawana, F., Shigeta, S., De Clercq, E., 1985. Inhibitory effects of several antiviral compounds on the replication of respiratory syncytial virus in vitro. *Antiviral Res. Suppl.* 1, 83–88.
- Kawana, F., Shigeta, S., Hosoya, M., Suzuki, H., De Clercq, E., 1987. Inhibitory effects of antiviral compounds on respiratory syncytial virus replication in vitro. *Antimicrob. Agents Chemother.* 31, 1225–1230.
- Kelscy, R., Alher, D., Bithell, S., Budworth, J., Carter, M., Chubb, A., Cockrill, S., Dowdell, V., Harland, R., Henderson, E., Keegan, S., McNamara, D., Strahles, J.N., Powell, K., Weerasekera, N., Wilson, L., 2004. Characterisation and mode of action for A-60444, a small molecule inhibitor of RSV. In: *Proceedings of the 44th International Conference on Antimicrobial Agents and Chemotherapy, Programs and Abstracts*. Abstract F-488.
- Kim, H.W., Canchola, J.G., Brandt, C.D.S., Pyles, G., Chanock, R.M., Jensen, K., Parrott, R.H., 1969. Respiratory syncytial virus disease in infants despite prior administration of antigenic inactivated vaccine. *Am. J. Epidemiol.* 89, 422–434.
- Kimura, K., Mori, S., Tomita, K., Ohno, K., Takahashi, K., Shigeta, S., Terada, M., 2000. Antiviral activity of NMSO3 against respiratory syncytial virus infection in vitro and in vivo. *Antiviral Res.* 47, 41–51.
- Krusat, T., Sueckert, H.J., 1997. Heparin-dependent attachment of respiratory syncytial virus (RSV) to host cells. *Arch. Virol.* 142, 1247–1254.
- Leaman, D.W., Longano, F.J., Okicki, J.R., Soike, K.F., Torrence, P.F., Silverman, R.H., Cramer, H., 2002. Targeted therapy of respiratory syncytial virus in African green monkeys by intranasally administered 2–5 Å antisense. *Virology* 292, 70–77.
- Leaman, D.W., 2005. 2–5 Å Antisense treatment of respiratory syncytial virus. *Curr. Opin. Pharmacol.* 5, 502–507.
- Leung, A.K., Kellner, J.D., Davies, H.D., 2005. Respiratory syncytial virus bronchiolitis. *J. Natl. Med. Assoc.* 97, 1708–1713.
- Lin, C.-C., Yeh, L.-T., Vitarella, D., Hoog, Z., 2003. Viramidine, a prodrug of ribavirin, shows better liver-targeting properties and safety profiles than ribavirin. *Antiviral Chem. Chemother.* 14, 145–152.
- Lizzi, M., Mason, S.W., Carrier, M., Lawetz, C., McCollum, R.S., Danstereau, N., Bolger, G., Lapeyre, N., Gaudette, Y., Tagace, L., Massariol, M.J., Do, F., Whitehead, P., Lamarre, L., Scutten, E., Bordeleau, J., Landry, S., Rancourt, J., Fazal, G., Simoes, B., 2005. Inhibitors of respiratory syncytial virus replication target transcriptional mRNA granulation by viral RNA-dependent RNA polymerase. *J. Virol.* 79, 13105–13115.
- Luchetti, M., Ferrero, F., Gallini, C., Natale, A., Pigna, A., Tortorolo, L., Marraro, G., 2002. Multicenter, randomized, controlled study of porcine surfactant in severe respiratory syncytial virus-induced respiratory failure. *Pediatr. Crit. Care Med.* 3, 261–268.
- Maggon, K., Burik, S., 2004. New drugs and treatment for respiratory syncytial virus. *Rev. Med. Virol.* 14, 149–168.
- Markland, W., McQuaid, T.J., Jain, J., Kwong, A.D., 2000. Broad-spectrum antiviral activity of the IMP dehydrogenase inhibitor VX-497: a comparison with ribavirin and demonstration of antiviral additivity with alpha interferon. *Antimicrob. Agents Chemother.* 44, 859–866.
- McHutchison, J.G., Shiffman, M.L., Cheung, R.C., Gordon, S.C., Wright, T.L., Potage Jr., J.C., McNair, L., Erte, B., Mosley, S., Alam, J., 2005. A randomized, double-blind, placebo-controlled dose-escalation trial of merimepodib (VX-497) and interferon-alpha in previously untreated patients with chronic hepatitis C. *Antiviral Ther.* 10, 635–643.
- McIntosh, K., Kurachek, S.C., Cairns, L.M., Burns, J.C., Goodspeed, B., 1984. Treatment of respiratory syncytial virus infection in an immunodeficient infant with ribavirin aerosol. *Am. J. Dis. Child.* 138, 305–308.
- Mejias, A., Chavez-Bueno, S., Rios, A.M., Aten, M.F., Raynor, B., Peromingo, F., Soni, P., Olson, K.D., Kiener, P.A., Gomez, A.M., Jafri, H.S., Ramilo, O., 2005. Comparative effects of two neutralizing anti-respiratory syncytial virus (RSV) monoclonal antibodies in the RSV murine model: time versus potency. *Antimicrob. Agents Chemother.* 49, 4700–4705.
- Mitsui, A., Suzuki, S., 1969. Immunosuppressive effect of mycophenolic acid. *J. Antibiot.* 22, 358–363.
- Nasr, S.Z., Strouse, P.J., Soskolne, E., Maxvold, N.J., Garver, K.A., Rubin, B.K., Moler, F.W., 2001. Efficacy of recombinant human deoxyribonuclease I in the hospital management of respiratory syncytial virus bronchiolitis. *Chest* 120, 203–208.
- Ni, L., Zhao, L., Qian, Y., Zhu, J., Jin, Z., Chen, Y.W., Tien, P., Gao, G.P., 2005. Design and characterization of human respiratory syncytial virus entry inhibitors. *Antiviral Ther.* 10, 833–840.
- Plantrose, D.N., 1969. Antiviral and cytotoxic effects of mycophenolic acid. *J. Gen. Virol.* 4, 629–630.
- PREVENT Study Group, 1997. Reduction of RSV hospitalization among premature infants and infants with bronchopulmonary dysplasia using RSV immune globulin prophylaxis. *Pediatrics* 99, 93–99.
- Prince, G.A., Mathews, A., Curtis, S.J., Porter, D.D., 2000. Treatment of respiratory syncytial virus bronchiolitis and pneumonia in a cotton rat model with systemically administered monoclonal antibody (palivizumab) and glucocorticosteroid. *J. Infect. Dis.* 182, 1326–1330.
- Razinkov, V., Huntley, C., Ellestad, G., Krishnamurthy, G., 2002. RSV entry inhibitors block F-protein mediated fusion with model membranes. *Antiviral Res.* 55, 189–200.
- Rixon, H.W.M., Brown, G., Murray, J.T., Sugrue, R.J., 2005. The respiratory syncytial virus small hydrophobic protein is phosphorylated via a mitogen-activated protein kinase p38-dependent tyrosine kinase activity during virus infection. *J. Gen. Virol.* 86, 375–384.
- Roberts, N.A., Appleton, R., Scott, R., 2001. Celcept (the prodrug of mycophenolic acid) is active against RSV disease in mice. *Antiviral Res.* 50, A76.
- Robinson, P.J., Hegels, R.G., Schellenberg, R.R., 1996. Increased airway reactivity in human RSV bronchiolitis in the guinea pig is not due to increased wall thickness. *Pediatr. Pulmonol.* 22, 248–254.
- Rodriguez, W.J., Arrojo, J., Fink, R., Kim, H.W., Milburn, C., 1999. Prospective follow-up and pulmonary functions from a placebo-controlled randomized trial of ribavirin therapy in respiratory syncytial virus bronchiolitis. Ribavirin Study Group. *Arch. Pediatr. Adolesc. Med.* 153, 469–474.

- Siddiqui-Jain, A., Grand, C.L., Bearss, D.J., Hurley, L.H. 2002. Direct evidence for a G-quadruplex in a promoter region and its targeting with a small molecule to repress c-MYC transcription. *Proc. Natl. Acad. Sci. U.S.A.* 99, 11593–11598.
- Sidwell, R.W., Bailey, K.W., Wong, M.H., Barnard, D.L., Smee, D.F. 2005. In vitro and in vivo influenza virus-inhibitory effects of viraquine. *Antiviral Res.* 68, 10–17.
- Sidwell, R.W., Huffman, J.H., Khare, G.P., Allen, L.B., Witkowski, J.T., Robins, R.K. 1972. Broad-spectrum antiviral activity of Virazole: 1- β -D-ribofuranosyl-1,2,4-triazole-3-carboxamide. *Science* 177, 705–706.
- Sidwell, R.W. 1996. Ribavirin: a review of antiviral efficacy. In: Pandalai G. (Ed.), *Recent Research and Development in Antimicrobial Agents and Chemotherapy*, vol. 1. Research Signpost, Kerala, India, pp. 219–256.
- Sudo, K., Watanabe, W., Mori, S., Konno, K., Shigeta, S., Yokota, T. 1999. Mouse model of respiratory syncytial virus infection to evaluate antiviral activity in vivo. *Antiviral Res.* 10, 135–139.
- Sudo, K., Miyazaki, Y., Kojima, N., Kobayashi, M., Suzuki, H., Shintani, M., Shimizu, Y. 2005. YM-53403, a unique anti-respiratory syncytial virus agent with a novel mechanism of action. *Antiviral Res.* 65, 125–131.
- Taber, L.H., Knight, V., Gilbert, B.E., McClung, H.W., Wilson, S.Z., Norton, H.J., Thurston, J.M., Gordon, W.H., Atmar, R.L., Schauldt, W.R. 1983. Ribavirin aerosol treatment of bronchiolitis associated with respiratory syncytial virus infection in infants. *Pediatrics* 72, 613–618.
- Techapornkul, S., Barretto, N., Peeples, M.F. 2001. Functional analysis of recombinant respiratory syncytial virus deletion mutants lacking the small hydrophobic and/or attachment glycoprotein gene. *J. Virol.* 75, 6825–6834.
- Thompson, W.W., Shay, D.K., Weintraub, E., Brammer, L., Cox, N., Anderson, L., Fukuda, K. 2003. Mortality associated with influenza and respiratory syncytial virus in the United States. *JAMA* 289, 179–186.
- Torrence, P.F. 1999. 2–5 A-antisense chimeras: inhibitors of respiratory syncytial virus infection. *Curr. Opin. Mol. Ther.* 1, 307–315.
- Weiss, W.J., Murphy, T., Lynch, M.E., Frye, J., Buklan, A., Gray, B., Lenoy, E., Mitelman, S., O'Connell, J., Quaruccio, S., Huntley, C. 2003. Inhalation efficacy of RFI-641 in an African green monkey model of RSV infection. *J. Mod. Primatol.* 32, 82–88.
- Wilson, L., Alber, D., Carter, M., Hadad, R., Henderson, E., Dowdell, V., Kelsey, R., Harris, R., Keegan, S., McNamara, D., Bithell, S., Weerasakera, N., Budworth, J., Chubb, A., Stables, J.N., Cockerill, S., Powell, K. 2004. Characterisation and mode of action for A-60444; a novel small molecule inhibitor of RSV. In: *Proceedings of the 44th Interscience Conference on Antimicrobial Agents and Chemotherapy, Programs and Abstracts*. Abstract P-489.
- Wong, J.Y., Moon, S., Beardsmore, C., O'Callaghan, C., Simpson, H. 2000. No objective benefit from steroids inhaled via a spacer in infants recovering from bronchiolitis. *Eur. Respir. J.* 15, 388–394.
- Woods, J.M., Marr, C.L.P., Penn, C.R. 1994. Inhibition of respiratory syncytial virus replication in vitro by a pyrazole dicarboxamide analogue of ribavirin. *Antiviral Chem. Chemother.* 5, 340–343.
- Wu, H., Pfaff, D.S., Tang, Y., An, L.L., Patel, N.K., Wulkins, J.D., Huse, W.D., Kiener, P.A., Young, J.F. 2005. Ultra-potent antibodies against respiratory syncytial virus: effects of binding kinetics and binding valence on viral neutralization. *J. Mol. Biol.* 350, 126–144.
- Wyde, P.R., Wilson, S.Z., Petrella, R., Gilbert, B.E. 1987. Efficacy of high-dose-short-duration ribavirin aerosol in the treatment of respiratory syncytial virus-infected cotton rats and influenza B virus-infected mice. *Antiviral Res.* 7, 211–220.
- Wyde, P.R., Gilbert, B.E., Ambrose, M.W. 1989. Comparison of the anti-respiratory syncytial virus activity and toxicity of papaverine hydrochloride and pyrazofurin in vitro and in vivo. *Antiviral Res.* 11, 15–26.
- Wyde, P.R., Ambrose, M.W., Meyer, H.L., Zolinski, C.L., Gilbert, B.E. 1990a. Evaluation of the toxicity and antiviral activity of carbocyclic 3-deazaadenosine against respiratory syncytial virus and parainfluenza type 3 viruses in tissue culture and in cotton rats. *Antiviral Res.* 14, 215–226.
- Wyde, P.R., Ambrose, M.W., Meyer, H.L., Gilbert, B.E. 1990b. Toxicity and antiviral activity of LY253963 against respiratory syncytial and parainfluenza type 3 viruses in tissue culture and in cotton rats. *Antiviral Res.* 14, 237–247.
- Wyde, P.R., Moore-Poveda, D.K., De Clercq, E., Neyts, J., Matsuda, A., Minakawa, N., Guzman, E., Gilbert, B.E. 2000. Use of cotton rats to evaluate the efficacy of antivirals in treatment of measles virus infections. *Antimicrob. Agents Chemother.* 44, 1146–1152.
- Wyde, P.R., Chetty, S.N., Timmerman, P., Gilbert, B.E., Andrics, K. 2003. Short duration aerosols of INJ 2408068 (R170591) administered prophylactically or therapeutically protect cotton rats from experimental respiratory syncytial virus infection. *Antiviral Res.* 60, 221–231.
- Wyde, P.R., Lequere, S., Chetty, S.N., Gilbert, B.E., Nitz, T.J., Pevsner, D.C. 2005. Antiviral efficacy of VP14637 against respiratory syncytial virus in vitro and in cotton rats following delivery by small droplet aerosol. *Antiviral Res.* 68, 18–26.
- Xu, Z., Kuang, M., Okicki, J.R., Cramer, H., Chaudhery, N. 2004. Potent inhibition of respiratory syncytial virus by combination treatment with 2–5 A antisense and ribavirin. *Antiviral Res.* 61, 196–206.

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REVIEW



New drugs and treatment for respiratory syncytial virus

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SUMMARY

The respiratory syncytial virus (RSV) is a global health problem affecting infants and the elderly and claiming more lives than AIDS in many parts of the world. Only two antibody drugs are approved for its prevention, and ribavarin, a relatively nonspecific antiviral, is used for treatment. In the mid-1990s, a number of pharmaceutical and biotech companies initiated research programs against RSV. Together, the academic and the industrial R&D covered the whole spectrum of antibodies, vaccines, synthetic small molecule antiviral and antisense technology, and at one point, accounted for at least 25 active R&D programs. However, coincident to the marketing of the monoclonal antibody palivizumab (Synagis[®]) in 1998, a sharp decline in such projects ensued. Many companies recently cancelled RSV projects during a prioritisation of their R&D portfolios although the continuing medical need, large market size and sales projections clearly indicate that a safe and effective RSV drug or vaccine is likely to attain blockbuster status. Today RSV receives an insignificant fraction of the R&D budget compared with AIDS, for example. This article reviews the present status of the anti-RSV regimen, covers drugs in the market and in development, and attempts to link basic research to industrial drug development, animal models of RSV, clinical trials, current clinical management, and present and future market projections. It is hoped that the unmet medical need of the victims of RSV will encourage continued involvement of the pharmaceutical and biotechnology industry in developing safe and effective prevention and treatments for RSV. Copyright © 2004 John Wiley & Sons, Ltd.

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INTRODUCTION

Human respiratory syncytial virus is the most common worldwide cause of lower respiratory tract infections (LRI) in infants less than 6 months of age and premature babies less than or equal to 35 weeks of gestation [1–4]. The RSV disease

spectrum includes a wide array of respiratory symptoms from rhinitis and otitis media to pneumonia and bronchiolitis, the latter two diseases being associated with considerable morbidity and mortality. Infants who are premature or have chronic lung disease or congenital heart disease are at particular risk for severe RSV disease. Although traditionally regarded as a pediatric pathogen, RSV can also cause life-threatening pulmonary disease in bone marrow transplant recipients and the elderly. It infects up to 65% of babies in the first year of life, essentially 100% within the first 2 years, and remains the main cause of bronchiolitis. In a recent World Health Organization (WHO) report [4], the global annual infection figure for RSV is estimated to be 64 million. In the USA it is estimated that RSV directly causes 18 000–75 000 hospitalisations and up to 1900 deaths annually, and contributes to another 17 000 deaths [1–4]. If the recent CDC data [3] are extrapolated

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Abbreviations used

AIDS, acquired immunodeficiency syndrome; AS-ODN, antisense oligodeoxynucleotide; CDC, Centers for Disease Control and Prevention; cp, cold-passaged; FDA, Food and Drug Administration; IND, investigational new drug; LRI, lower respiratory tract infection; NDA, new drug application; NIAID (NIH), National Institute of Allergy and Infectious Diseases (National Institutes of Health); NSAID, non-steroidal anti-inflammatory drug; PFP, purified fusion protein; R&D, research and development; RSV, respiratory syncytial virus; RSV-IGIV, RSV immune globulin intravenous; ts, temperature-sensitive; WHO, World Health Organization

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globally, then the number of elderly victims of RSV, which may be four times higher than infants, would total 2.4 to 4 million per year. Overall, RSV is a major killer worldwide, claiming 3–5 million human lives annually. Not surprisingly, WHO has designated RSV as a major target of research and therapy throughout the globe [4].

A number of features of RSV have contributed to the difficulties of prevention and treatment. First of all, RSV has an RNA genome, and all RNA genomes accumulate mutations at a high rate due to the lack of replicational proof-reading mechanisms, which presents a significant challenge in designing a reliable vaccine or antiviral [6–8]. Second, the 15 kb viral genome codes for at least 11 proteins, some of which are unique and of unknown physiological role [9]. Third, RSV is rather difficult to grow in cell cultures and is prone to losing its infectivity. Fourth, it interacts with cells and specific cellular proteins, adding to the difficulty of obtaining a cell-free viral material [10–13]. Fifth, RSV–host interactions promise to involve a large and complex network of signaling pathways that must play important roles in the manifestation of the RSV disease, but are only beginning to be unraveled [14–23]. Finally, the immunopathology of RSV is equally complex [4,23–26], and includes the relatively unique phenomenon of vaccine-enhanced disease or ‘immunopotential’ [27–30]. In short, RSV exemplifies many intriguing challenges of an antiviral regimen.

In December 2002, the FDA approved Integrated Biotechnology Corporation’s application to market the QuickLab™ RSV kit for rapid qualitative detection of the presence of RSV in nasopharyngeal aspirates (www.integratedbiotech.com). The test was approved for children aged 6 years and younger, and adults aged 60 and over. Three other rapid commercial tests were found to have low predictive value with a 10%–23% success rate [31]. Clearly, a low-cost, sensitive, specific and rapid test is an obligatory first step towards mapping out the RSV disease pattern and epidemiology, particularly in the developing world.

VIRAL GENOME AND RELEVANT GENES

RSV is a member of the genus *Pneumovirus* in the family *Parainflaviridae*. The single-stranded negative-sense RNA genome is tightly wrapped with the viral protein N to form the

nucleocapsid [9,32]. The genome encodes three transmembrane surface proteins (F, G, SH), one matrix protein (M), the nucleocapsid protein (N), nucleocapsid-associated proteins (M2–1, P, L), a M2–2 protein (the second product of the M2 gene) and two nonstructural proteins (NS1, NS2). The viral envelope is composed of a plasma membrane-derived lipid bilayer that contains virally encoded transmembrane proteins. The minimal viral RNA-dependent RNA polymerase comprises the large protein L and the phosphoprotein P, but requires viral M2–1 (22K) protein as well as cellular actin and profilin for optimal activity [9,11–13]. The surface fusion (F) and attachment (G) glycoproteins are the major, if not the only, viral components that induce RSV neutralising antibody, and thus, constitute important targets of vaccine development [33–36]. Both proteins are heavily glycosylated with an interesting difference: while the F protein is primarily N-glycosylated on asparagine residues, G is O-glycosylated on serines and threonines [33–35]. The glycosylations in G are located in the proximal and distal domains that have a mucinoid structure, whereas the central domain contains a highly conserved cysteine-rich region. Studies with knockout RSV mutants have clearly shown viral penetration and fusion with the target cell without the involvement of G and SH proteins, and studies with RSV F expressed in other enveloped virus vectors have also documented fusion and entry with F alone [33].

Cross-neutralisation studies have shown that RSV isolates can be classified into two subgroups, designated A and B [7,8], that exhibit antigenic and genetic differences and can be further segregated into virus lineages or clades. Although the sequences of all 11 viral proteins differ to one extent or another between A and B strains, the G glycoprotein shows the greatest divergence, with only 53% amino acid identity between prototype A and B viruses [34,35]. Group A and B can co-circulate during epidemics, although one may predominate. Both A and B groups have been further classified into subgroups, and several investigators have reviewed the regional and global epidemiology of these strains [7,8,23,37]. The impact of antigenic diversity on RSV epidemiology is not completely understood, but may in part explain the susceptibility to reinfection throughout life and the yearly variation in the severity of epidemics within communities [7,8,37].

THE RSV DISEASE

RSV epidemics occur annually during winter and early spring in temperate climates and during the rainy season in some tropical climates [1–4]. Humans are the only known reservoir for RSV, although specific RSV strains also exist for nonhuman animals such as cattle, sheep and goats. As mentioned before, premature infants, immunocompromised adults or children with bronchopulmonary dysplasia are at increased risk for acquiring severe RSV disease (pneumonia and bronchiolitis), often requiring hospitalisation. Spread of this highly contagious virus via contaminated nasal secretions requires close contact with an infected individual or contaminated environmental surface. By 2 years of age, almost all children get infected with RSV at least once and about half of them, twice [1–4,38–41]. Overall, roughly 25%–40% of RSV-infected young adults and healthy older children develop lower respiratory tract infections (LRI) with many developing bronchiolitis or pneumonia [38–42]. Reinfection can occur throughout life and is usually symptomatic.

IMMUNOLOGY OF RSV DISEASE

Virus-specific immune responses are largely responsible for protection against RSV-associated LRI and recovery from RSV infection. Immunity to RSV is mediated via humoral and cellular effectors, including serum antibody (acquired as a result of infection or maternally derived in young infants), secretory antibody and major histocompatibility complex class I- and class II-restricted cytotoxic T lymphocytes [1,2,5,25,26,38–42]. Epidemiological studies and viral challenge in healthy young adults have confirmed that the local antibody response to RSV is short-lived, and reinfection occurs throughout life. Moreover, RSV-associated LRI can occur in young children experiencing their second episode of RSV. The lack of protection may be explained in part by group specificity, since children with primary RSV infection develop a neutralising antibody response in serum more frequently and of greater magnitude to the infecting strain than to a heterologous RSV strain. In general, humoral immune responses involving secretory antibodies and serum antibodies appear to protect against infection of the upper and lower respiratory tract, respectively, while cell-mediated responses directed against internal proteins appear to terminate infection [38–42].

The role of local immunity in the protection of the upper respiratory tract against RSV is suggested by the observation that passive transfer of neutralising antibodies offers protection but does not significantly affect viral replication in the upper respiratory tract [5,25,38–42]. Cotton rats that were given live RSV via the respiratory tract were resistant to re-challenge for 6 to 12 months, suggesting that local immune responses inhibited reinfection. In adult volunteers, the presence of secretory neutralising antibody, but not serum antibody, correlated with protection of the upper respiratory tract. In infants, the development of RSV-specific immunoglobulin A (IgA) in nasal secretions correlated temporally with disease clearance.

RSV replicates primarily in the respiratory epithelium. This may explain why serum-neutralising antibody does not prevent infection, as it does for pathogens that produce viremia, such as measles and varicella-zoster viruses. However, high titers of serum neutralising antibody against RSV do prevent LRI by RSV, as has been demonstrated in animal studies and epidemiological observations, although a spectrum of results has been observed in clinical trials of RSV hyperimmune globulin (RSV-IGIV), RespiGam[®], in high-risk infants [43–50]. High titers of maternally derived RSV antibody, as measured by IgG enzyme-linked immunosorbent assay (ELISA) or neutralisation assay in maternal blood or cord blood, have been shown to correlate inversely with the incidence of RSV infection and the severity of RSV pneumonia in the first 6 months of life. In young children, the rate of reinfection with RSV and the rate of LRI at the time of reinfection also correlate inversely with the level of serum neutralising antibody against RSV achieved after the primary infection. Finally, a randomised, prospective study of administration of RSV-IGIV to high-risk infants showed significant reductions in the rate and severity of LRI, the need for hospitalisation and days spent in intensive care [43,44,47], although the effect on the duration of hospital stay was either small [47] or insignificant [44]. The use of RSV-IGIV after the onset of RSV infection did not offer any clinical benefit [47]. Interestingly, the titer of RSV neutralising antibody achieved in infants who received this dose of RSV-IGIV was comparable to that demonstrated to protect the lungs of cotton rats against RSV infection. The protective effect of

RSV-IGIV in these young infants was confirmed by subsequent placebo-controlled trials. Although the prophylactic efficacy of RSV-IGIV has been established, it does not appear to ameliorate RSV disease when given therapeutically.

Young infants develop levels of neutralising antibody and F and G glycoprotein antibodies that are only 15 to 25% of those observed in older children [40,41]. Such studies also demonstrate that the serum IgA response to the F glycoprotein is affected primarily by age, while the response to the G glycoprotein is affected primarily by the pre-existing RSV IgG titer. The suboptimal response of young infants to primary RSV infection has important implications for vaccine development, since it suggests that more than one dose of vaccine will probably be needed to induce adequate levels of RSV neutralising antibody in this population. Finally, as indicated earlier, cell-mediated immunity appears to be important in the termination of RSV infection [41,42]. Studies with mice suggest that antibodies are not essential to the clearance of the virus. Children with defects in cell-mediated immunity and athymic or gamma-irradiated mice can shed virus indefinitely. Lastly, lung epithelial cells mount a strong inflammatory response to RSV infection by elaborating a wide variety of

cytokines and chemokines that further amplify the inflammation process.

IMMUNOGLOBULINS AND MONOCLONALS FOR IMMUNOPROPHYLAXIS OF RSV

Table 1 lists the anti-RSV immunoglobulin and monoclonal antibodies that are either in the market or under active development. Currently, RespiGam[®] (respiratory syncytial virus immune globulin intravenous, human; RSV-IGIV) and Synagis[®] (palivizumab) are the only two antibodies prescribed in RSV disease (Table 1) [43–46, 48–58]. RSV-IGIV is an intravenous (IV) polyclonal immune globulin enriched in neutralising antibodies against RSV [51]. Developed by MedImmune and marketed by Wyeth and Baxter for the prevention of serious RSV in high-risk infants, it was shown to reduce hospitalisation by 41%, hospital stay by 53%, and total RSV-related intensive care unit days by 44% [51]. RSV-IGIV is given by a 2–4 h IV infusion, and a course of treatment costs about \$5000 per infusion [51,55,59,60]. In the 1997–1998 RSV seasons, market sales were \$70 million [59]; however, sales have declined to negligible levels as it has been replaced by palivizumab.

Palivizumab is a humanised anti-F monoclonal antibody (IgG1 κ) composed of human (95%)

Table 1. Anti-RSV antibodies in market and under development

Product	Characteristics	Company	Sales 2003/Status	Ref./Patent
RespiGam (RSV-IGIV)	Immunoglobulin	MedImmune ¹	Negligible	[50,51,55]
Synagis (Palivizumab)	hAnti F-glycoprotein	MedImmune ¹	\$849 M	[45,46,48–54,56–59]
Felvizumab* RSHZ19	hAnti F-glycoprotein	GSK ² Scotgen	Phase III completed	[45,61–64] WO 0069462
HNK20* MoAb	Immunoglobulin	CSL/Oravax ³ Acambis ⁴	Phase III completed	[126] US 5534411
Numax	Monoclonal antibody	MedImmune	Phase I	US 201404 US 5824307
R19	Monoclonal antibody	EpicYTE ⁵	Preclinical	[45] WO 0183806

The Company/Agency world-wide web sites are: ¹*medimmune.com* (also: *synagis.com*, *rsveducation.com*); ²*gsk.com*; ³*csl.com.au*; ⁴*acambis.co.uk*; ⁵*epicYTE.com*. In all Tables, the patents are listed by their numbers in the USA, Europe (EP) or World Intellectual Property Organization (WIPO) administered Patent Cooperative Treaty PCT (WO). All tables include compounds that were in development by the industry at the end of 3Q 2003 and listed as active in Pharma Projects or Prous Science. Compounds discovered in the Universities/Research Institutes or at NIAID/NIH (except for the NIH vaccine) were only included if licensed to the industry. In all Tables, asterisks (*) indicate compounds that are no longer found under the active R&D projects at the company web sites and thus, were considered discontinued in 2003.

New drugs for RSV

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and murine (5%) antibody sequences. Designed by Dainippon and licensed to MedImmune (Gaithersburg, MD), palivizumab received US approval in 1998 for the prevention of serious LRI by RSV [52]. It is the current market leader in RSV therapy; annual sales were \$670 million in the year 2002 and \$849 million in 2003 [51]. Palivizumab has become the standard for prevention of RSV in high-risk infants such as premature ones and those with chronic lung and congenital heart diseases. This recombinant antibody is manufactured in cell culture to high purity and yield, and neutralises a broad range of RSV strains in animal models. In a phase III clinical study in 1502 high-risk infants, it reduced the incidence of hospitalisations by 55% [48]. Another double-blind randomised placebo-controlled study in 1287 children with congenital heart disease over 4 years (i.e. four RSV seasons) showed a 45% reduction in hospitalisations in the treated group [56]. Palivizumab can be administered by a short-term IV or IM infusion, and a liquid formulation for easier use may be available in 2004 if approved by the FDA. Palivizumab is 50–100 times more potent than RSV-IGIV (RespiGam) and is now approved in over 50 countries. Several clinical studies of palivizumab deserve mention: five studies in adult volunteers ($n=38$), four prophylaxis studies in pediatric subjects ($n=1281$), and five treatment studies in both adult and pediatric patients ($n=75$) [45,46,48–54,56–59]. Overall, the five studies in adult volunteers showed that the half-life of elimination of palivizumab was approximately 17–20 days when administered either IV or IM, and that it was safe through at least three monthly injections (with the uppermost test dose of 15 mg/kg). Because RSV-IGIV is a polyimmunoglobulin, some of its benefit could be attributed to non-RSV immunoglobulin. Understandably, such 'carrier' immunoglobulin effects were not observed in palivizumab trials. Post-marketing safety surveillance of palivizumab in more than 250 000 high risk infants over a 4-year period (1998–2002) confirmed its safety and clinical effectiveness [57].

MedImmune has recently submitted an Investigational New Drug (IND) application to FDA for NumaxTM, an affinity-matured monoclonal antibody created by Applied Molecular Evolution, a San Diego-based start up company. It is claimed to be 20 times more active than palivizumab in reducing RSV viral loads in cell cultures and in lungs of cotton rats [51].

A few years ago, Scotgen RSHZ19 (Fulvizumab) was at the same stage of development as RespiGam (RSV-IGIV) and was in fact ahead of palivizumab [45,61–64]. However, its development was probably delayed initially due to licensing deals with SmithKline Beecham (SKB) and later due to the merger between SKB and Glaxo Wellcome to create Glaxo Smith Kline (GSK). Phase III trials have since been completed but the results are not available. The immunoglobulin HNK20 progressed to phase III trials; however, there is no mention of it in the current company web site (Acambis). Its development was probably delayed by the licensing deal from the originator Australian start up company CSL to Oravax, an American biotechnology start up, followed by takeover of Oravax by the UK-based Acambis. Another immunoglobulin preparation (named Sandoglobulin[®]), extracted from human plasma by ZLB Central Laboratory (Switzerland) and distributed by Novartis as intravenous and aerosol formulations, failed to show any efficacy [47,65]. A review of US patents revealed that although IDEC Pharmaceuticals Corporation (San Diego, CA) had filed and obtained several patents for a monoclonal RSV antibody from 1995 to 2002 (USP 6413771), the RSV program (AV 2921) is no longer listed as active on the company web site.

In conclusion, the current experience with RSV-IGIV and palivizumab suggests that appropriately formulated and administered immunoprophylactic therapy of severe RSV disease can be safe and effective.

VACCINES

Although the importance of RSV as a respiratory pathogen has been recognised for over 40 years and several vaccines have been tested since, a reliable one is yet to come by, in part due to several inherent problems described earlier. The search for a vaccine has indeed been the most active area of RSV research, especially since early studies showed adverse reactions following RSV vaccination, leading to the recognition of an ill-understood phenomenon called 'immunopotential' or 'vaccine-enhanced disease' [24–30]. In brief, an experimental formalin-inactivated RSV vaccine (Pfizer Lot 100) tested in children in the mid 1960s was immunogenic but failed to protect against RSV infection. Indeed, the immunised children, whose age was consistent with the vaccine

Table 2. RSV vaccines under development

Product	Characteristics	Target population	Company/Agency	Status/Phase	Ref./Patent
LA	Cpts 248/404	Infants	Wyeth ¹ NIAID ²	II	[70,71]
LA	A2cpts 248/404-SH	Infants	Wyeth NIAID	II	[72-74]
LA	RB/HPIV3-RSV-A/B	Infants	MedImmune	P	[90] US 6565849
LA	RA2, M2-2	Infants	NIAID	P	[66]
LA	2B33F	Infants	NIAID	P	[66]
SV	PFP1	Elderly	Wyeth	I	[66-69]
SV	PFP2	Elderly	Wyeth	II	[77,79]
SV	PFP3	Elderly	Wyeth	II	[78]
SV	BBG2Na	Elderly	Pierre Fabre Medicament ³	III	[81,82] EP 1353690 WO 03010317
SV	FG487808*	Elderly	CSK	I	[84]; WO 0228426
SV	F + C	Elderly	Aventis ⁴	II	[83]; WO 0035481

LA, live attenuated; SV, subunit vaccine; P, preclinical. The Company/Agency world-wide web sites are: ¹wyeth.com; ²niaid.gov; ³cipf.com; ⁴roventis.com; other addresses and patent codes are provided in Table 1. Vaccines such as vaccinia adenovirus (Adf), human rhinovirus (DDJFV3) and cpts B176 are not listed due to lack of adequate information.

being the first exposure to RSV antigens, experienced a more severe disease in subsequent RSV infection compared with those that received placebo. Immunopotentiality was observed with denatured protein or killed virus, the major factors being altered T cell sensitisation [24-30] and significant contributions from immune complexes [27-30]. Subsequent studies showed that the antibody response to the F protein was lower in the immunised patients than in the control, and the antibodies were in fact non-neutralising. To sum up, the currently accepted hypothesis for immunopotentiality is that denatured RSV epitopes, in contrast to natural RSV infection, generate nonprotective antibodies and an undesirable T-helper response. Another major issue regarding RSV vaccines is the immunologic immaturity of the newborn babies and infants and the declining immune system of the elderly. Moreover, an RSV vaccine must protect against the antigenically divergent groups, A and B. As serious RSV disease can occur in high-risk individuals with a previous history of RSV infection as well as in RSV-naïve infants, it is also likely that more than one type of RSV vaccine will be needed to immunise all who would benefit from vaccination.

Nonetheless, the success of immunoprophylaxis points to the prospects of a preventive vaccine. A number of vaccines in fact progressed to animal

models and toxicology studies and some to phase I and II clinical trials, but all eventually circled back to the discovery and formulation stages for optimisation of the adjuvant or boosting of the immunogenicity, thereby starting the development clock once again under current regulations [36,66-70]. In the past 20 years of RSV vaccinology, two main types of vaccines were formulated: live attenuated virus, primarily for young infants [70] and subunit vaccines for the elderly and the older RSV-seropositive children with chronic cardiac or pulmonary disease [69]. Progress in these areas is summarised below and in Table 2.

Live attenuated vaccines

Several strategies for the development of a live attenuated (weakened) RSV vaccine were explored, including the creation of host range mutants, cold-passaged (cp) mutants, and temperature-sensitive (ts) mutants [71-76]. In brief, these vaccine candidates were either under-attenuated (cpRSV and RSVts-1) or over-attenuated (RSVts-2). The weakened strains are expected to be avirulent and not cause a productive infection or illness, although the possibility of illness in immunocompromised persons has not been ruled out. Wyeth tested cpts 248/404 in RSV seronegative and seropositive children in a double-blind, randomised and placebo-controlled study. The vaccine was

immunogenic and attenuated when given intranasally and caused mild to moderate upper respiratory congestion when administered to 1–2 month old RSV naïve infants. It was considered reasonably safe but not safe enough to permit further development [73–75]. Recent constructs of rA2 cp 248/404 SH and rA2 cp 248/404-11030 SH, when tested in 4–12 week old infants, did not show any nasal congestion and was immunogenic. The rB/IPIV3-RSV A and B are considered promising vaccines in animal model studies but must overcome regulatory safety concerns about introducing a chimeric recombinant virus to newborns. Transmission of the ts-1 mutant from vaccinated children to placebo recipients was in fact found to occur. Further information can be found in the NIAID Jordan report [66] and a review by Simoes [67] that covered 22 phase I–II studies with various vaccines up to mid-2001. Some recent publications of Piedra, Wyeth and Pierre Fabre Medicament [36,69–71] provide an up-to-date summary of current vaccines under clinical testing.

Subunit vaccines

Although vector delivery systems, synthetic peptide, DNA and immune-stimulating complex vaccines were evaluated in animal models, subunit vaccines such as those based on purified F protein (PFP) have shown the greatest promise. PFPs were evaluated in phase I–II clinical trials with demonstrated safety and immunogenicity [77–79]; PFP2, in particular, appeared to be safe for the elderly and for RSV-seropositive children with underlying pulmonary disease. A phase I study in 35 healthy third trimester pregnant women showed safe and efficient transplacental transfer of RSV neutralising antibodies to infants [79]. However, it was only modestly immunogenic compared with RSV. A phase II double-blind controlled study of the PFP3 subunit vaccine in cystic fibrosis children showed the vaccine to be safe and immunogenic; however, it failed to reduce the incidence of LRI in this study [78]. The French company, Pierre Fabre Medicament, formulated BBG2Na, a subunit G vaccine and demonstrated its safety, tolerance and immunogenicity in young adults [80–82]. The immunogenicity and safety of the BBG2Na in a targeted elderly population under phase II trial was considered sufficient to warrant further development [69,81,82]. Phase III studies were initiated in 2000 and have been completed since,

but the results are not publicly available. The Aventis vaccine consists of F, G and M subunits and has completed two phase I studies in healthy adults where it was found to be safe and immunogenic [83]. An FG chimeric vaccine is in early development [84].

In summary, the highly complex immunopathology of the RSV disease has so far foiled attempts to develop a safe and effective vaccine. The reinfection observed despite the presence of viral antibodies has not been overcome. Current regulations in the USA and Europe make it very difficult to test subunit vaccines in newborn babies. The very high safety and efficacy bar placed on the vaccines for premature babies pushes development costs and timelines.

ANTIVIRAL COMPOUNDS

Antivirals are either serendipitous natural compounds or rationally designed drugs that are based on our knowledge of the molecular biology of the virus and/or host–virus interactions. Although a need for nonimmunological alternatives against RSV is appreciated, the antiviral area has received relatively little support, and most of the compounds have never crossed the preclinical hurdles [85–106]. Several early-stage antivirals for RSV are listed in Table 3.

Ribavirin (Virazole[®]), the most inexpensive RSV drug, is a broad-spectrum antiviral agent [85–90]. It inhibits viral multiplication by several mechanisms, i.e. inhibition of viral polymerase, inhibition of 5' cap formation of mRNA, and inhibition of IMP dehydrogenase leading to a decrease of intracellular GTP levels. The only antiviral RSV to win FDA approval, ribavirin is marketed by Valeant Pharmaceuticals (formerly ICN). However, its clinical benefits are small and occur only in a fraction of RSV-infected patients; thus, its use is now limited mainly to immunocompromised patients or to early treatment of severe RSV. Ribavirin spray (aerosol) reduces the severity of the disease and the amount of virus load, without reducing the duration of hospitalisation [88–90].

Several antiviral compounds were screened but found to be ineffective against RSV. A few polysaccharides blocked entry of the virus into host cells; however, they were not active against cells already infected with the virus, as expected. The beta-agonist adrenergic agents such as albuterol

Table 3. RSV antivirals

Product	Nature	Company	Sales/ Status	Ref./Patent
Ribavarin/Virazole	Antiviral	Valeant ¹	\$50 M	[8,88–90]
Provir/Virend	Plant Flavonoid	Inverni	Phase III	[103,104]
Crofelemer*		Della Beffa ²		
RFI 641*	Triazine	Wyeth	Phase II	[96–98] US 5852015
VP 14637*		ViroPharma ³	Phase I	[105]; US 6495580
A 60444	Antiviral	Arrow ⁴	Phase I	
MBX 3000	Antiviral	Microbiotix ⁵	D	[112]; US 2002077472
RSV therapy	Antiviral	Biota ⁶	D	[106]; WO 03040178
Antiviral*	Triphenol	Gilead ⁷	D	
BMS 4333771	Benzimidazole	Bristol-Myers Squibb ⁸	D	WO0004900 US 2002016309
Antisense	2–5A	Topigen ⁹	D	WO 03014153
RSV Therapy		Apath ¹⁰	D	
Fusion protein*	Benzathrone	Trimeris ¹¹	D	[107]; WO 0292575
CL 309623*	Triazine	Wyeth	D	EP 0795549, US 5852015
MP 351*	2–5A antisense	Manhattan Pharma ¹²	P	[115,116]
RD 30028*	Benzoditin	Kuraray ¹³	P	[101,102]; US 5698580
R 170591	Benzimidazole	Design Lab Janssen ¹⁴ J&J ¹⁵	P	[108,109]; EP 1196408 WO 0100611
J&J 2408068				

The Company/Agency world-wide web sites are: ¹valeant.com (also see virazole.com, rsvinfo.com); ²giofil.it; ³viropharma.com; ⁴arrowt.co.uk; ⁵microbiotix.com; ⁶biota.com.au; ⁷gilead.com; ⁸bms.com; ⁹topigen.com; ¹⁰apath.com; ¹¹trimeris.com; ¹²manhattanpharma.com; ¹³kuraray.co.jp; ¹⁴janssenpharmaceutica.be; ¹⁵nj.com; others are provided in Tables 1 and 2. D = lead Discovery or lead optimisation stage; P = Preclinical compound in animal models. Many of these compounds have been subsequently discontinued.

have shown only minimal or no improvements of respiratory symptoms in clinical studies. Anticholinergic drugs (such as ipratropium) and corticosteroids (budesonide) failed to show any clinical benefit in the treatment of RSV bronchiolitis [91–93]. Chinese herbal extracts such as anagyrrine, oxymatrine, sophoranol, wogonin, oroxylin A, undecic acid A and B were observed to have potent anti-RSV activity [94–95]. Recently, a novel polynuclear aromatic compound, dubbed RFI-641, was studied for its anti-RSV activity [96–98] and seemed to act by inhibiting F-mediated fusion of the virus. In another report [99] a cysteinyl-leukotriene receptor antagonist reduced lung symptoms subsequent to RSV bronchiolitis. Infants ($n = 130$), aged 3–36 months and hospitalised with acute RSV bronchiolitis, were randomised into a

double-blind placebo-controlled study of 5 mg montelukast (Merck Sharp & Dohme Ltd) chewable tablets for 28 days starting up to 7 days after symptom debut. Those on montelukast were free of any symptoms on 22% of the days and nights, compared with 4% of the days and nights in those on placebo ($p = 0.015$).

RSV infection activates NF- κ B as well as other transcription factors that in turn activate a plethora of pro-inflammatory cytokines and chemokines, such as IL-1, IL-8, RANTES [14,15,18,21,100]. Non-steroidal anti-inflammatory drugs (NSAID) of the salicylate family, such as aspirin, were shown to inhibit the activation of NF- κ B by RSV and thereby inhibit the activation of multiple cytokines in the RSV-infected cell [100]. MedImmune was granted a patent (WO 0182966) for the use of NSAID

with immunoprophylactic agents. Nevertheless, concerns about gastrointestinal and hepatic toxicity of NSAIDs and drug interactions with antivirals remain to be addressed.

Several early-stage antiviral compounds are listed in Table 3 and include the plant flavanoid provir, VP 14637 and benzoditin RD3 0028 [101–107]. Bristol-Myers Squibb's benzimidazole derivative provided 100% protection in HEp-2 cells infected with RSV at a concentration of 4 µg/ml versus 100% protection by ribavirin at 2.5 µg/ml. The benzimidazole analog of Johnson & Johnson showed potent anti-RSV activity in cell cultures and animal models [108,109]. In cell culture studies, Abbott reported inhibition of RSV by diacetyl tartaric acid mono and diglycerides (DATEM) and Nissin reported inhibition with a sulfated sialyl lipid (NMSO3) [109–111]. Enzimidazole from Trimeris inhibited membrane fusion-associated events including syncytium formation with 50% inhibition at 0.01 µg/ml [112]. All the compounds listed in Table 3 that are in the discovery and preclinical stages claimed high and selective viral inhibitory activity in RSV-infected HEp2 cells and exhibited low cytotoxicity. Polyoxometalate (Triangle Pharma) and Benzoditin-RD3 (Kuraray) were recently discontinued. Antisense antivirals are reviewed below.

ANTISENSE DRUGS

Commonly used antisense molecules are oligodeoxynucleotides (AS-ODNs) that are 15–20 nucleotide long synthetic DNA molecules complementary to small segments of the target mRNA. The antisense compounds have the advantage of being less complex and potentially quicker and more efficient than traditional drugs directed towards protein targets [113,114]. Modified AS-ODNs in which various numbers of phosphodiester bonds have been substituted with phosphorothioate linkage are often preferred for their stability against nucleases present in cells and biological fluids; however, phosphorothioate ODNs may have a lower sequence specificity and higher toxicity. The dominant mechanism of AS-ODN activity involves binding of the ODN to the complementary RNA to produce a DNA-RNA hybrid, followed by degradation of the RNA strand by RNase H. However, as RNase H is primarily a nuclear enzyme, this mechanism is not applicable to cytoplasmic RNA viruses such as RSV.

Two alternative mechanisms have, therefore, been exploited against RSV. The first one involves AS-ODNs designed against the translational regulatory sequences of the mRNA (such as ribosome-binding site) so that a direct inhibit of translation is achieved without degrading the mRNA. Such antisense ODNs, targeted against the two nonstructural proteins, NS1 and NS2, achieved a substantial abrogation of the corresponding proteins (S. B., unpublished observation; US patent 5831 069). Although the effects of the anti-NS ODNs on RSV are yet to be fully evaluated, it is assumed that they will have little or no toxicity since the NS sequences have no orthologs in any other species. This was borne out by cell culture studies in which no obvious cytopathic effect was observed in uninfected A549 cells. The phosphorothioate oligos are highly stable as dry powder, can be reconstituted with water, and can be routinely synthesised in a standard DNA synthesiser. Hybridon used AS-ODNs against viral genomic RNA, but discontinued them at the preclinical stage. About 80% viral inhibition was achieved at a relatively high concentration of 1 µM ODN, and 90% inhibition, at 10 µM. The lower effectiveness of these AS-ODNs could be due to the fact that their target, i.e. the genomic RNA, is tightly covered with nucleocapsid protein. The AS-ODNs of Genta and Novopharm were in early stages of research when discontinued. In general, there are other technical problems with AS-ODNs that still need to be overcome. Delivery to the target cells is one, and analogs of DNA and RNA are being investigated to improve stability. Attaining a sufficiently large intracellular concentration without overt cellular toxicity is another challenge [114]. To sum up, almost all antisense compounds for RSV are now discontinued, and it appears that the corresponding resources have been shifted to cancer and related areas.

In an interesting second mechanism, AS-ODN are coupled to 2',5'-oligoA, which recruits cellular 2',5'-oligoA-dependent RNase L, resulting in the degradation of the target viral RNA [115,116]. NIH and the Cleveland Clinic had licensed this technology to a start up drug company (Manhattan Pharmaceuticals, formerly Atlantic) for development and marketing. This is difficult chemistry and may not be suitable for automation. These oligos are also relatively unstable and less effective. At a high concentration of 3.3 µM, 80% viral

inhibition, and at 7.5 μM , 90% inhibition was achieved. The clinical development of this strategy remained unclear and the project was terminated.

RNA INTERFERENCE AS ANTIVIRAL

The antiviral effect of RNA interference was first demonstrated against RSV [117] and has been subsequently reproduced against a number of other viruses, including HIV [118,119]. The mechanism co-opts an evolutionary conserved cellular RNA degradative pathway, commonly called 'RNA interference' (RNAi) and found in all eukaryotes [120–122]. In RSV, for example, specially designed double-stranded RNA (dsRNA) corresponding to specific viral mRNA sequences successfully knocked down the targeted mRNAs and produced the expected mutant phenotypes. Thus, ablation of RSV fusion protein (F) abolished syncytium formation in cell culture. Double-stranded RNA longer than 30 base pairs tends to elicit an 'interferon response' that shuts down general translation of capped mRNAs [122]. The short length (21–22 nt) of the anti-RSV dsRNAs ruled out such global inhibition, and allowed for specific abrogation of target RNA without undue toxicity to the host [117,120]. This is a new and emerging area that holds enormous potential but needs further research for clinical development.

DEVELOPMENT AND TESTING OF RSV DRUGS

In this section we will provide a few representative examples of the RSV-related assays that are commonly conducted for drug testing. All new drugs for RSV must undergo extensive preclinical testing *in vitro* and *in vivo* (animal models) as well as toxicology studies, followed by clinical trials of safety and efficacy. For monoclonal antibodies, the NDA (New Drug Application) profile of palivizumab can serve as a good model [52,53]. For RSV vaccines, the development program is similar, but with more emphasis on safety and extensive clinical trials as in the case of FluMist™ (Influenza Virus Vaccine Live, Intranasal) (MedImmune, Wyeth). For new antiviral drugs, an extensive program of safety and efficacy studies in animals should be developed and executed after consultation and approval of FDA for IND filing. For all types of RSV drugs, it is recommended that studies be done in close cooperation with NIAID.

In vitro binding/neutralisation assays

(a) Micro-neutralisation assay: In the case of palivizumab, for example, an ELISA-based micro neutralisation assay is performed to detect virus replication using the Long strain of RSV; the parental MAb 1129 is used as a control. (b) Plaque reduction/neutralisation assay: Dilutions of the test drug are employed against RSV Long (A subtype) and against RSV 18537 (B subtype). Palivizumab or ribavirin is often used as the positive control. The number of plaque forming units (pfu) is assayed on susceptible cell lines such as HEP-2, and compared with palivizumab. In recent studies, investigational drugs were tested against a panel of 57 clinical isolates from different geographic areas of the USA/Europe, using the ELISA-based micro neutralisation assay [46,108–112].

In vivo assays (animal models of RSV)

In the mouse model for antivirals the RSV load is administered intranasally followed by treatment on days 1–7 and assessment of viral load and lung pathology on days 4 or 8. The dosing and assessment vary with vaccine and antibodies [61,69,81,102,123–126].

The cotton rat (*Sigmodon hispidus*) model is semi-permissive for RSV infection. Clinical symptoms are not observed after infection and passive transfer of antibodies is reasonably predictable. FDA approval of RSV-JGIV is based on the cotton rat model as the only preclinical model for the antibody. Nonetheless, the predictability of the cotton rat model with regard to vaccines must be cautiously interpreted [27,51,69,84,101,103,123–126].

The virus is inoculated into the nares of the animals and the resulting viral load and pathology noted in the lungs and upper airways. Infected animals develop bronchiolitis and focal pneumonia. A variety of treatment times such as before infection and at different days post infection can be tested to define the best dosing option. The effective dose is commonly defined as 100-fold reduction in viral load in the lungs. Additional studies should explore and define efficacy upon primary versus secondary infection.

The minipig model has been used occasionally. African green monkey (no real clinical symptoms) and chimpanzee (only rhinorrhea observed, no LRI symptoms) are used in the later phases of drug trial due to their higher costs; they help in making the decision to go to clinical studies [69]. Rhesus

monkey and African green monkey models are closer to humans for testing vaccines, monoclonal antibodies, antiviral and antisense drugs [76,96,123,126]. The unavailability and the high cost of primates are serious drawbacks and discourage further research, validation or routine use of the monkey models [69,71]. The chimpanzee, in particular, is a protected species. Smaller New World primates such as the owl, spider, cebus or squirrel monkeys, are potential alternatives but would require scientific and regulatory validation and acceptance for routine use in RSV studies. Bonnet monkeys and infant baboons are also under investigation as RSV infection models.

Effect of RSV infection on serum drug levels

Four to six cotton rats are IV-dosed with the study drug, followed by challenge 24 h later with 10^5 pfu of RSV Long strain. Sera levels of the drug are measured using an ELISA assay specific for human IgG. Sera levels should be similar for uninfected and infected animals.

Biological half-life

The pharmacokinetic (PK) profiles of proteins from individual or pooled bioreactor samples are evaluated. Animals (generally cotton rats; six/gender/group) are dosed with the study drug, and samples collected up to 96 h. Evaluation of samples via a sandwich ELISA provides an idea of the pharmacokinetics in the rats. It can also offer information about batch-to-batch variability and any biochemical or glycosylation differences as they may determine the half-life or potency of the drug.

PK/ADME (pharmacokinetic/absorption-distribution-metabolism-excretion)

Once the antiviral activity is established, it is common to conduct a single dose PK study in the cynomolgus monkeys that have been prescreened to be free of RSV-specific antibodies. A single dose is administered intravenously, followed by blood sampling up to 20 days post-dose. Clinical pathology is determined by comparing baseline and day 21 findings.

Toxicology

The exact logistics of toxicology would depend largely on the frequency and dosage of the investigational drug. For vaccines that may be administered

as only one or two injections per year or antibodies that may be used only once a month or so, only limited single and multiple dose toxicity studies are required. In contrast, antiviral compounds that may be meant for daily use require more extensive toxicology studies. The sponsor generally performs safety studies in monkeys, rabbits and cotton rats. Drug administration into animals by different routes in single or multiple doses must not result in any overt clinical or histopathological toxicity. Additionally, cross-reactivity of the drug is tested by incubation with frozen normal cynomolgus monkey tissue specimens (kidney, liver, skin, heart, small intestine). Finally, human tissue cross-reactivity is ruled out using several human adult and neonatal tissues. Particularly extensive toxicology studies are required for low molecular weight antiviral compounds due to their greater ability to spread systemically and cross blood-organ barriers.

Clinical antiviral trials

The phase I trials are dose-ranging and are designed to establish the pharmacokinetic profile of the drug. The test drug at 3–5 doses is tested in six healthy adult subjects per dose group. The blood levels and plasma elimination half-life is determined and correlated with the data from cotton rats and other animal models. Doses higher than the therapeutic dose are tried to establish the highest safe and effective dose. For vaccines, phase I studies also aim to establish the safety and immunogenicity. The moral and ethical dilemma of testing new drugs on premature babies is very difficult to resolve for the parents as well as the medical profession. The initial clinical testing is, therefore, done in RSV-infected elderly and adults.

The phase II trials differ depending on the type of drug. For antivirals, the first efficacy trials are done in immunocompromised adult patients such as the elderly or the transplant recipients suffering from RSV infections. As a rule, regulatory bodies prefer double-blind placebo-controlled trials enrolling at least 60–80 patients per arm. Efficacy parameters include nasal and throat swabs along with lung lavages to titer RSV, to determine the time of elimination of virus and complications of the disease and safety aspects. The subsequent study can then be done in RSV-infected babies.

For vaccines, antisense and immunoprophylactic drugs the phase II trials can be started in patients at risk of serious disease and the reduction in the rate of RSV related hospitalisations can be monitored. Secondary criteria would include complications during hospitalisations such as the risk of LRI, ventilation etc. The initial study can be done in infants or adult patients. Post vaccination surveillance for disease associated with wild type RSV infection is a necessary component of all RSV vaccine trials in seronegative children; in phase I and II trials, the primary purpose is to monitor for enhanced disease, and in phase III trials, to assess protection against RSV-associated LRI. The use of placebo-controlled, double-blind trials with post-vaccination surveillance through RSV epidemics is the model for future evaluation of live attenuated vaccines in children.

The phase III protocol design is similar to the phase II trial except that the number of patients is in the 3000–5000 range for antibodies and antivirals and 12000–60000 for vaccines. As very few hospitals have such a large number of patients, these are generally global pivotal IND studies over nearly a hundred sites and requiring large resources. A double-blind comparative study with Synagis alone and in combination with the investigative drug may be required for regulatory and marketing purposes. The clinical development schedule and trial design should be discussed with and approved by the FDA and/or the EMEA prior to initiating the trials.

RSV DRUG DEVELOPMENT AND THE PHARMACEUTICAL INDUSTRY

The final step in bringing a drug from the bench to the bedside is the realm of the pharmaceutical industry, and therefore, we would like to end this review with an evaluation of this area of RSV drug development and the outlook for the future. Information provided in the previous sections should leave little doubt that RSV presents a potentially huge drug market. In the USA alone, 3.5–4 million infants acquire RSV infection per year, resulting in nearly 75000 hospitalisations, and an equal number is attributed to Europe. Overall, the worldwide market could easily reach 5–10 million newborn babies. In addition, there are 60000 elderly RSV-related hospitalisations per year in the USA. The cost of treating a high-risk

child hospitalised for RSV in the USA can exceed \$70000 [1–5,64,127]. Thus, a safe and effective new treatment targeted at infants and the elderly could bring in \$5 billion per year at peak sales for each category. Within 4 years of marketing, palivizumab has already achieved sales of \$670 million in the year 2002 and \$849 million in 2003. If the current rate of over 25% sales growth continues, palivizumab sales may top \$1 billion in 2004 [48]. It is difficult to estimate the contribution of RSV-related sales to the worldwide total ribavirin sales of \$1.4 billion (in the year 2001); nonetheless, estimates are in the \$50–75 million range. Clearly, an effective and safe drug, developed for prophylaxis can reach a blockbuster status of over several billions of dollars. Each of the live-attenuated RSV vaccines for infants and the subunit vaccines for the elderly could reach sales of \$1 billion, provided that they are safe and effective.

In apparent contrast, practically all large pharmaceutical companies have either eliminated or greatly curtailed their anti-infective research activities in the past 4 years [128–130]. Such names include Aventis, Bristol-Myers Squibb, Eli Lilly, GlaxoSmithKline, Proctor & Gamble, Roche and Wyeth, and it is fair to assume that many others will follow suit. The problem was epitomised by a session titled 'Why is big pharma getting out of anti-infective drug discovery?' in the 2003 ICAAC meeting in Chicago, in which Dr Steve Projan, a Wyeth R&D vice-president, was the keynote speaker. A plethora of reasons, summarised under Conclusion, contributed to the disinterest; however, the primary disincentive is financial. In the USA, the Institute of Medicine recently issued a report to find new ways to finance vaccine development [131].

The contribution of industrial R&D, which covers the whole spectrum of antibodies, specific monoclonal antibodies, vaccines, synthetic small molecule antiviral and antisense technology to control RSV, is listed in Tables 1–3. About 7 years ago, there were at least 25 active R&D programs. However, coincident to the marketing of the immunoglobulin palivizumab in 1998, there has been a significant decline in such projects. Note that almost all antisense projects have now been discontinued and several vaccines were discontinued at phase I–II stage. Only four projects, a subunit G vaccine (BBG2Na), an immunoglobulin (HINK20), an antiviral (Provir) and a monoclonal

antibody (Felvizumab), moved to phase III in Europe after several years in phase I/II trials. Phase III trials were also completed by early 2003; however, the results have not been released, obviously signifying a discouraging outcome.

At this time, only MedImmune and NIAID (NIH) continue to attach a high priority to RSV vaccines, the first to maintain, protect and expand its current market dominance of RSV treatment. The extensive use of palivizumab in the USA (95% of global sales) compared with Europe (5% of global sales in 2002 and 2003) is probably dictated more by a relatively lower potential for litigation, a lack of other safe and effective alternatives, and an intelligent marketing strategy. MedImmune involved almost all the major hospitals in the USA and Europe in their extensive phase III trials, thus creating market advocates. Palivizumab is claimed to have prevented RSV infection in over 500 000 infants [50,58,127], which is a significant achievement that also contributed to its huge commercial success. Palivizumab cuts down hospitalisation rates by almost 50%; ironically, the other 50% constitute the empty half of the glass, paying a high price without deriving significant benefits from the treatment. Thus, there is certainly room for improvement in terms of efficacy, safety and formulation. The liquid formulation, due in 2004, promises to have greater heat stability and ease of administration. In general, most of the current pharmaceutical efforts focus on the development of humanised monoclonal antibodies for passive immunisation, recombinant cDNA-based vaccines such as live attenuated, subunit G vaccine and purified fusion protein FFP for primary immunisation and annual booster.

CONCLUSION: QUO VADIS?

It appears that three major reasons have contributed to the dampened enthusiasm in the anti-RSV industry: the inherent immunological challenges of RSV, the high-risk nature of the patient populations, and ultimately, economic factors. The vaccines and the drugs for both groups of patients—the elderly and the infants—must overcome major safety hurdles besides efficacy and still perform under the specter of expensive litigations. Our understanding of the RSV immunopathology in natural infection as well as in relation to vaccines and antibody therapy must become more comprehensive. The cost of drug development in general

has reached astronomic proportions, currently estimated at \$1.7 billion. Pharmaceutical production of the drug in a GMP facility may cost an additional \$400 million. Time is another hindrance; the development of a new vaccine typically takes over 10 years, and clinical trials require 12 000–45 000 individuals enrolled in a 2:1 ratio in the treatment and the placebo groups.

An equally important factor is public awareness. Much has been written about the politics of 'body-count' funding and orphan diseases, and it has been argued that the extraordinary awareness and resources devoted to AIDS, which is a rather easily preventable disease, owes a great deal to the AIDS activists and the popular media [132–134]. Whether this is true or not, there is no denying that the past decade has indeed witnessed major advances in AIDS drug regimens and treatments including fast track approvals, reduced costs and user-friendly protocols, all translating into real benefits to patients. Since RSV strikes at the starting and the terminal phases of life, patients are unlikely to take any activist role or form pressure groups. The RSV advocacy group of the future will likely emerge from the parents of infants or adults who have lost their parents or grandparents to RSV. Nonetheless, we would argue that RSV deserves serious attention on all counts. As regards body count, every 10 s one human dies as a result of RSV infection and another is infected. However, body count alone often does not reveal the total burden on the population. The staggering cost of RSV-related health care has been described above in the section on RSV Drug Development and The Pharmaceutical Industry; the lost time of working parents further adds to the cost. Scientific pursuits and scholarly endeavors also sometimes suffer from lack of awareness or attention, with a ripple effect on public education. For example, although the antiviral effect of RNA interference was first reported in RSV [117], it received little [135] or no [136] mention in some recent reviews, whereas essentially similar effects on other viruses such as HIV were highly trumpeted.

There is another factor that compounds the problem but often goes unappreciated. The two major RSV patient groups—namely the infants and the elderly—dwell at the extreme ends of the age spectrum, each with its own unique immunopathological, pharmacological and social issues. The infants have a naïve immune system, whereas the elderly

have an aging body with multiple age-related diseases. Thus, one vaccine or drug may not work optimally for both. The industry, on the other hand, is generally reluctant to develop multiple regimens for what is conceived as the same disease because it reduces and fragments the market projections and sales, while nearly doubling the cost.

As we have indicated, existing therapies for RSV-infected patients are few, and efficacies are questionable at best. The greatest success so far has resulted from the use of humanised antibodies, and there is little reason to assume that the current status will change unless there is a major new breakthrough. Unfortunately, the potential future compounds that are under development and tabulated in this review have also shown limited efficacy in animal models so far. A combination of immunoprophylactic (vaccine) and therapeutic (antiviral) approaches might be our best overall strategy to control RSV in a population. We speculate that the strongest anti-RSV regimens of the future will evolve from a combination of immunological (multiple vaccines, humanised antibodies, interferon) and molecular biological (antisense, RNAi, rational drugs, natural compounds) agents. In the USA, the FDA may consider granting a fast track designation to the prevention and treatment of RSV. At the end, however, an international consortium of governments, collaborative laboratories, philanthropic individuals and agencies, free reagent banks, and a certain amount of camaraderie, sacrifice and public education may be needed to stop the RSV disease from attaining an orphan status [137]. Precedence already exists for successful multi-pronged initiatives in a number of other neglected diseases such as malaria and tuberculosis [138]. The Foundation for the NTH, established by the US Congress, indeed identifies and develops opportunities for innovative public-private partnerships (www.fnih.org), some of which can be channeled to RSV drug development.

INFORMATION RESOURCES FOR RSV DRUGS

Consulting readily available FDA/EMEA sources on the Internet to find unpublished data on anti-RSV drugs can lead to a better understanding of drug safety and efficacy. The FDA/EMEA website includes detailed summaries and assessments of the actual data submitted in support of the NDA.

Reviews of pre-clinical studies and clinical trials submitted to the FDA/EMEA are available [49,51,52]. The materials distributed to the FDA Advisory Committee members before approval, including summaries of the FDA reviews, are now posted on the internet the day before the Advisory Committee meeting (<http://www.fda.gov/ohrms/dockets/ac/00mtbc.htm>). The transcripts of these meetings are valuable sources of information for new drugs under review (<http://www.fda.gov/foi/electr.htm>). Similar information is available on the Internet for public scrutiny for the accepted IND files of several new vaccines like FluMist (Influenza virus vaccine live, intranasal). The NIH (NIAID), CDC, Institute of Medicine and Public Citizen web sites offer valuable information about the disease and its treatment and the list of ongoing trials in RSV [66,131]. MedImmune and Valeant [51,88] web sites offer prescription information and other details about the currently approved drugs. The commercial web sites of Pharma Projects, Prous Science, Current Drugs, Scrip, BioCentury also remain important sources for drug R&D. BiomedCentral and PubMed offer free access to many full text papers and abstracts. In writing this review, we have consulted and cited references and patents as recent as December 2003 from the ISI web of Science, Science Direct, PubMed, WIPO, US and European Patent Office. To keep the number of references reasonable, recent reviews or papers have often replaced original research papers and thus, we sincerely apologise to the many researchers whose work could not be cited.

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REFERENCES

- Welliver RC. Respiratory syncytial virus and other respiratory viruses. *Pediatr Infect Dis J* 2003; 22: S6-S12.

2. Greenough A. Respiratory syncytial virus infection: clinical features, management, and prophylaxis. *Curr Opin Pulm Med* 2002; 8: 214–217.
3. Thompson WW, Shay DK, Weintraub E, et al. Mortality associated with influenza and respiratory syncytial virus in the United States. *JAMA* 2003; 289: 179–186.
4. http://www.who.int/vaccine_research/documents/new_vaccines/en/index2.html
5. Brandenburg AH, Neijens HJ, Osterhaus AD. Pathogenesis of RSV lower respiratory tract infection: implications for vaccine development. *Vaccine* 2001; 19: 2769–2782.
6. Domingo E, Holland JJ. RNA virus mutations and fitness for survival. *Annu Rev Microbiol* 1997; 51: 151–178.
7. Sullender WM. Respiratory syncytial virus genetic and antigenic diversity. *Clin Microbiol Rev* 2000; 13: 1–15.
8. Crotty S, Andino R. Implications of high RNA virus mutation rates: lethal mutagenesis and the antiviral drug ribavirin. *Microbes Infect* 2002; 4: 1301–1307.
9. Collins PL, Camargo E, Hill MG. Support plasmids and support proteins required for recovery of recombinant respiratory syncytial virus. *Virology* 1999; 259: 251–255.
10. Barik S. Transcription of human respiratory syncytial virus genome RNA *in vitro*: requirement of cellular factor(s). *J Virol* 1992; 66: 6813–6818.
11. Burke E, Dupuy L, Wall C, Barik S. Role of cellular actin in the gene expression and morphogenesis of human respiratory syncytial virus. *Virology* 1998; 252: 137–148.
12. Burke E, Mahoney NM, Almo SC, Barik S. Profilin is required for optimal actin-dependent transcription of respiratory syncytial virus genome RNA. *J Virol* 2000; 74: 669–675.
13. Bitko V, Oldenburg A, Garmon NE, Barik S. Profilin is required for viral morphogenesis, syncytium formation, and cell-specific stress fiber induction by respiratory syncytial virus. *BMC Microbiol* 2003; 3: 9.
14. Mastronarde JG, He B, Monick MM, Mukaida N, Matsushima K, Hunninghake GW. Induction of interleukin (IL)-8 gene expression by respiratory syncytial virus involves activation of nuclear factor (NF)-B and NF-IL-6. *J Infect Dis* 1996; 174: 262–267.
15. Bitko V, Barik S. Persistent activation of RelA by respiratory syncytial virus involves protein kinase C, underphosphorylated I κ B β , and sequestration of protein phosphatase 2A by the viral phosphoprotein. *J Virol* 1998; 72: 5610–5618.
16. Mastronarde JG, Monick MM, Mukaida N, Matsushima K, Hunninghake GW. Activator protein-1 is the preferred transcription factor for cooperative interaction with nuclear factor-kappaB in respiratory syncytial virus-induced interleukin-8 gene expression in airway epithelium. *J Infect Dis* 1998; 177: 1275–1281.
17. Chen W, Monick MM, Carter AB, Hunninghake GW. Activation of ERK2 by respiratory syncytial virus in A549 cells is linked to the production of interleukin 8. *Exp Lung Res* 1999; 26: 13–26.
18. Fiedler MA, Wernke-Dollries K. Incomplete regulation of NF-kappaB by I κ B α during respiratory syncytial virus infection in A549 cells. *J Virol* 1999; 73: 4502–4507.
19. Casola A, Garofalo RP, Haerberle H, et al. Multiple cis regulatory elements control RANTES promoter activity in alveolar epithelial cells infected with respiratory syncytial virus. *J Virol* 2001; 75: 6428–6439.
20. Monick M, Staber J, Thomas K, Hunninghake G. Respiratory syncytial virus infection results in activation of multiple protein kinase C isoforms leading to activation of mitogen-activated protein kinase. *J Immunol* 2001; 166: 2681–2687.
21. Tian B, Zhang Y, Luxon BA, et al. Identification of NF-kappa B-dependent gene networks in respiratory syncytial virus-infected cells. *J Virol* 2002; 76: 6800–6814.
22. Carpenter LR, Moy JN, Roebuck KA. Respiratory syncytial virus and TNF alpha induction of chemokine gene expression involves differential activation of Rel A and NF-kappa B1. *BMC Infect Dis* 2002; 2: 5.
23. Hacking D, Hull J. Respiratory syncytial virus—viral biology and the host response. *J Infect* 2002; 45: 18–24.
24. Jafri HS. Role of chemokines in RSV disease. *Pediatr Infect Dis J* 2002; 21: 454–456.
25. Varga SM, Braciale TJ. RSV-induced immunopathology: dynamic interplay between the virus and host immune response. *Virology* 2002; 295: 203–207.
26. Holt PG, Sly PD. Interactions between RSV infection, asthma, and atopy: unraveling the complexities. *J Exp Med* 2002; 196: 1271–1275.
27. Prince GA, Curtis SJ, Yim KC, Porter DD. Vaccine-enhanced respiratory syncytial virus disease in cotton rats following immunization with Lot 100 or a newly prepared reference vaccine. *J Gen Virol* 2001; 82: 2881–2888.
28. Openshaw PJ, Culley FJ, Olszewska W. Immunopathogenesis of vaccine-enhanced RSV disease. *Vaccine* 2002; 20: S27–S31.
29. Haynes LM, Jones LP, Barskey A, Anderson LJ, Tripp RA. Enhanced disease and pulmonary eosinophilia associated with formalin-inactivated respiratory syncytial virus vaccination are linked

- to G glycoprotein CX3C-CX3CR1 interaction and expression of substance P. *J Virol* 2003; 77: 9831–9844.
30. Polack FP, Teng MN, Collins PL, et al. A role for immune complexes in enhanced respiratory syncytial virus disease. *J Exp Med* 2002; 196: 859–865.
 31. Casiano-Colón AE, Hulbert BB, Mayer TK, Walsh EE, Falsey AR. Lack of sensitivity of rapid antigen tests for the diagnosis of respiratory syncytial virus infection in adults. *J Clin Virol* 2003; 28: 169–174.
 32. Banerjee AK, Barik S, De BP. Gene expression of nonsegmented negative strand RNA viruses. *Pharmacol Ther* 1991; 51: 47–70.
 33. Techaarpornkul S, Collins PL, Peeples ME. Respiratory syncytial virus with the fusion protein as its only viral glycoprotein is less dependent on cellular glycosaminoglycans for attachment than complete virus. *Virology* 2002; 294: 296–304.
 34. Teng MN, Collins PL. The central conserved cysteine noose of the attachment G protein of human respiratory syncytial virus is not required for efficient viral infection *in vitro* or *in vivo*. *J Virol* 2002; 76: 6164–6171.
 35. Sugawara M, Czaplicki J, Ferrage J, et al. Structure-antigenicity relationship studies of the central conserved region of human respiratory syncytial virus protein G. *J Pept Res* 2002; 60: 271–282.
 36. Piedra PA. Future directions in vaccine prevention of respiratory syncytial virus. *Pediatr Infect Dis J* 2002; 21: 482–487.
 37. Cane PA, Matthews DA, Pringle CR. Analysis of respiratory syncytial virus strain variation in successive epidemics in one city. *J Clin Microbiol* 1994; 32: 1–4.
 38. Weisman LE. Current respiratory syncytial virus prevention strategies in high-risk infants. *Pediatr Int* 2002; 44: 475–480.
 39. Wright PF, Gruber WC, Peters M, et al. Illness severity, virus shedding, and antibody responses in patients hospitalized with bronchiolitis caused by respiratory syncytial virus. *J Infect Dis* 2002; 185: 1011–1018.
 40. Crowe JE Jr. Influence of maternal antibodies on neonatal immunization against respiratory viruses. *Clin Infect Dis* 2001; 33: 1720–1727.
 41. Staat MA. RSV infections in children. *Semin Respir Infect* 2002; 17: 15–20.
 42. Openshaw PJM. Potential therapeutic implications of new insights into respiratory syncytial virus disease. *Respir Res* 2002; 3: S15–S20.
 43. Simoes EA, Soudheimer HM, Top FH Jr, et al. Respiratory syncytial virus immune globulin for prophylaxis against respiratory syncytial virus disease in infants and children with congenital heart disease: The Cardiac Study Group. *J Pediatr* 1998; 133: 492–499.
 44. Hemming VG, Rodriguez W, Kim HW, et al. Intravenous immunoglobulin treatment of respiratory syncytial virus infections in infants and young children. *Antimicrob Agents Chemother* 1987; 31: 1882–1886.
 45. Johnson S, Griego SD, Pfarr DS, et al. A direct comparison of the activities of two humanized respiratory syncytial virus monoclonal antibodies: MEDI-493 and RSHZ19. *J Infect Dis* 1999; 180: 35–40.
 46. Johnson S, Oliver C, Prince GA, et al. Development of a humanized monoclonal antibody (MEDI-493) with potent *in vitro* and *in vivo* activity against respiratory syncytial virus. *J Infect Dis* 1997; 176: 1215–1224.
 47. Rodriguez WJ, Gruber WC, Groothuis JR, et al. Respiratory syncytial virus immune globulin treatment of RSV lower respiratory tract infection in previously healthy children. *Pediatrics* 1997; 100: 937–942.
 48. The IMPact-RSV Study Group: palivizumab, a humanized respiratory syncytial virus monoclonal antibody, reduces hospitalization from respiratory syncytial virus infection in high-risk infants. *Pediatrics* 1998; 102: 531–537.
 49. Groothuis JR, Nishida H. Prevention of respiratory syncytial virus infections in high-risk infants by monoclonal antibody (palivizumab). *Pediatr Int* 2002; 44: 235–241.
 50. FDA 1996. NDA Product Approval Information RespiGam (Respiratory Syncytial Virus Immune Globulin Intravenous). <http://www.fda.gov/cber/products/rsvmas011896.htm>
 51. MedImmune Web Site: <http://www.medimmune.com>; RespiGam[®] and Synagis[®] Package Inserts.
 52. FDA 1998. BLA Product Approval Information: Synagis (Palivizumab). <http://www.fda.gov/cber/products/palimed061998.htm>
 53. EMEA European Public Assessment Report (EPAR) Synagis Revision 2, 12/11/02. <http://www.eudra.org/humandocs/Humans/EPAR/Synagis/Synagis.htm>
 54. Committee on Infectious Diseases and Committee on Fetus and Newborn, American Academy of Pediatrics, Policy Statement: revised indications for the use of palivizumab and respiratory syncytial virus immune globulin intravenous for the prevention of respiratory syncytial virus infections. *Pediatrics* 2003; 112: 1442–1446.
 55. PREVENT Study Group. Reduction of RSV hospitalization among premature infants and infants with bronchopulmonary dysplasia using RSV immune globulin prophylaxis. *Pediatrics* 1997; 99: 93–99.

56. Simoes EAF. Immunoprophylaxis of RSV: global experience. *Respir Res* 2002; 3: S26-S33.
57. Romero JR. Palivizumab prophylaxis of respiratory syncytial virus disease from 1998 to 2002: results from four years of palivizumab useage. *Pediatr Infect Dis J* 2003; 22: S46-S54.
58. Zambon M. Active and passive immunization against respiratory syncytial virus. *Rev Med Virol* 1999; 9: 227-236.
59. Kamal-Bahl S, Doshi J, Campbell J. Economic analyses of respiratory syncytial virus immunoprophylaxis in high-risk infants—a systematic review. *Arch Pediatr Adol Med* 2002; 156: 1034-1041.
60. Barton LL, Grant KL, Lemen RJ. Respiratory syncytial virus immune globulin: decisions and costs. *Pediatr Pulmonol* 2001; 32: 20-28.
61. Crowe JE Jr, Murphy BR, Chanock RM, Williamson RA, Barbas CF 3rd, Burton DR. Recombinant human respiratory syncytial virus (RSV) monoclonal antibody Fab is effective therapeutically when introduced directly into the lungs of RSV-infected mice. *Proc Natl Acad Sci USA* 1994; 91: 1386-1390.
62. Davis CB, Hepburn TW, Urbanski JJ, et al. Preclinical pharmacokinetic evaluation of the RSV-specific reshaped human monoclonal antibody RSHZ19. *Drug Metab Dispos* 1995; 23: 1028-1036.
63. Everitt DE, Davis CB, Thompson K, et al. The pharmacokinetics, antigenicity, and fusion-inhibition activity of RSHZ19, a humanized monoclonal antibody to respiratory syncytial virus, in healthy volunteers. *J Infect Dis* 1996; 174: 463-469.
64. Meissner HC, Groothuis JR, Rodriguez WJ, et al. Safety and pharmacokinetics of an intramuscular monoclonal antibody (SB209763) against respiratory syncytial virus (RSV) in infants and young children at risk for severe RSV disease. *Anticancer Agents Chemother* 1999; 43: 1183-1188.
65. Rimensberger PC, Burek-Kozłowska A, Morell A, et al. Aerosolized immunoglobulin treatment of RSV infection in infants. *Pediatr Infect Dis J* 1996; 15: 209-216.
66. National Institute of Allergy and Infectious Diseases NIAID/NIH. The Jordan Report. Accelerated development of vaccines. 2002. (<http://www.niaid.gov>).
67. Simoes EA, Tan DH, Ohlsson A, Sales V, Wang EE. Respiratory syncytial virus vaccine: a systematic overview with emphasis on respiratory syncytial virus subunit vaccines. *Vaccine* 2002; 20: 954-960.
68. Piedra PA. Clinical experience with respiratory syncytial virus vaccines. *Pediatr Infect Dis J* 2003; 22: S94-S99.
69. Power UF. Vaccine anti-VRS. Infections virales respiratoires tome 2. *Bronchopneumopathies Virales*, Freymuth F (ed.). Elsevier: Paris, 2001.
70. Murphy BR, Collins PL. Live attenuated virus vaccines for respiratory syncytial and parainfluenza viruses; applications of reverse genetics. *J Clin Invest* 2002; 110: 21-27.
71. Karron RA, Wright PF, Crowe JE Jr, et al. Evaluation of two live, cold-passaged, temperature-sensitive respiratory syncytial virus vaccines in chimpanzees and in human adults, infants, and children. *J Infect Dis* 1997; 176: 1428-1436.
72. Karron RA, Buonagurio DA, Georgiu AF, et al. Respiratory syncytial virus SH and G proteins are not essential for viral replication *in vitro*: clinical evaluation and molecular characterization of a cold-passaged, attenuated RSV subgroup B mutant. *Proc Natl Acad Sci USA* 1997; 94: 13961-13966.
73. Whitehead SS, Firestone CY, Karron RA, et al. Addition of a missense mutation present in the L gene of respiratory syncytial virus (RSV) cpts 530/1030 to RSV vaccine candidate cpts 248/404 increases its attenuation and temperature sensitivity. *J Virol* 1999; 73: 871-877.
74. Juhasz K, Whitehead SS, Boulanger CA, Firestone CY, Collins PL, Murphy BR. The two amino acid substitutions in the L protein of cpts 530/1009, a live-attenuated respiratory syncytial virus candidate vaccine, are independent temperature-sensitive and attenuation mutations. *Vaccine* 1999; 17: 1416-1424.
75. Wright PF, Karron RA, Belshe RB, et al. Evaluation of a live, cold-passaged, temperature-sensitive, respiratory syncytial virus vaccine in infancy. *J Infect Dis* 2000; 182: 1331-1342.
76. Jin H, Cheng X, Traina-Dorge VL, et al. Evaluation of recombinant respiratory syncytial virus gene deletion mutants in African green monkeys for their potential as live attenuated vaccine candidates. *Vaccine* 2003; 21: 3647-3652.
77. Groothuis JR, King SJ, Hogerman DA, Paradiso PR, Simoes EA. Safety and immunogenicity of a purified F protein respiratory syncytial virus (PFP-2) vaccine in seropositive children with bronchopulmonary dysplasia. *J Infect Dis* 1998; 177: 467-469.
78. Piedra PA, Cron SG, Jewell A, et al. Immunogenicity of a new purified fusion protein vaccine to respiratory syncytial virus: a multi-center trial in children with cystic fibrosis. *Vaccine* 2003; 21: 2448-2460.
79. Munoz FM, Piedra PA, Glezen WP. Safety and immunogenicity of respiratory syncytial virus purified fusion protein-2 vaccine in pregnant women. *Vaccine* 2003; 21: 3465-3467.
80. Plotnicky-Gilquin H, Robert A, Chevalet L, et al. CD4(+) T-cell-mediated antiviral protection of the upper respiratory tract in BALB/c mice following parenteral immunization with a recombinant

- respiratory syncytial virus G protein fragment. *J Virol* 2000; 74: 3455–3463.
81. Power UF, Nguyen TN, Rietveld E, *et al.* Safety and immunogenicity of a novel recombinant subunit respiratory syncytial virus vaccine (BBG2Na) in healthy young adults. *J Infect Dis* 2001; 184: 1456–1460.
 82. Power UF, Plotnicky H, Blaecke A, Nguyen TN. The immunogenicity, protective efficacy and safety of BBG2Na, a subunit respiratory syncytial virus (RSV) vaccine candidate, against RSV-B. *Vaccine* 2003; 22: 168–176.
 83. Sales V. Safety and immunogenicity of a respiratory syncytial virus subtype A (RSV-A) vaccine in adults—Two phase I studies. Presented at the IV International Symposium on respiratory viral infections, Curacao 2001; cited in Ison MG, Mills J, Openshaw P, Zambon M, Osterhaus A, Hayden F. Current research on respiratory viral infections: Fourth International Symposium. *Antiviral Res* 2002; 55: 227–278.
 84. Prince GA, Capiav C, Deschamps M, *et al.* Efficacy and safety studies of a recombinant chimeric respiratory syncytial virus FG glycoprotein vaccine in cotton rats. *J Virol* 2000; 74: 10287–10292.
 85. Prince GA. An update on respiratory syncytial virus antiviral agents. *Expert Opin Investig Drugs* 2001; 10: 297–308.
 86. Jafri HS. Treatment of respiratory syncytial virus: antiviral therapies. *Pediatr Infect Dis J* 2003; 22: S89–S93.
 87. Shigeta S. Recent progress in antiviral chemotherapy for respiratory syncytial virus infections. *Expert Opin Investig Drugs* 2000; 9: 221–235.
 88. Valeant Pharmaceuticals, Inc. 2003. Virazole® (ribavirin for inhalation solution) prescribing information (US). All about RSV and Physicians Guide: <http://www.valeant.com, virazole.com, rsvinfo.com>.
 89. Edell D, Khoshoo V, Ross G, Salter K. Early ribavirin treatment of bronchiolitis—Effect on long-term respiratory morbidity. *Chest* 2002; 122: 935–939.
 90. Guerguerian AM, Gauthier M, Lebel MH, Farrell CA, Lacroix J. Ribavirin in ventilated respiratory syncytial virus bronchiolitis: a randomized, placebo-controlled trial. *Am J Respir Crit Care Med* 1999; 160: 829–834.
 91. Chowdhury D, al Howasi M, Khalil M, al-Frayh AS, Chowdhury S, Ramia S. The role of bronchodilators in the management of bronchiolitis: a clinical trial. *Ann Trop Paediatr* 1995; 15: 77–84.
 92. Buckingham SC, Jafri HS, Bush AJ, *et al.* A randomized double-blind placebo controlled trial of dexamethasone in severe respiratory syncytial virus (RSV) infection: effects on RSV quantity and clinical outcome. *J Infect Dis* 2002; 185: 1222–1228.
 93. Richter H, Seddon P. Early nebulized budesonide in the treatment of bronchiolitis and the prevention of postbronchiolitic wheezing. *J Pediatr* 1998; 132: 849–853.
 94. Ma SC, Du J, But PP, *et al.* Antiviral Chinese medicinal herbs against RSV. *J Ethnopharmacol* 2002; 79: 205–211.
 95. Ma LY, Ma SC, Wei F, *et al.* Uncinoside A and B Two new antiviral chromone glycosides from *Selaginella Uncinata*: *Chem Pharm Bull (Tokyo)* 2003; 51: 1264–1267.
 96. Weiss WJ, Murphy T, Lynch ME, *et al.* Inhalation efficacy of RFI-641 in an African green monkey model of RSV infection. *J Med Primatol* 2003; 32: 82–88.
 97. Hunlley CC, Weiss WJ, Gazumyan A, *et al.* RFI-641, a potent respiratory syncytial virus inhibitor. *Antimicrob Agents Chemother* 2002; 46: 841–847.
 98. Aulabaugh A, Ding W, Ellestad G, *et al.* Inhibition of respiratory syncytial virus by a new class of chemotherapeutic agents. *Drugs of the Future* 2000; 25: 287–294.
 99. Bisgaard H. A randomized trial of montelukast in respiratory syncytial virus post-bronchiolitis. *Am J Respir Crit Care Med* 2003; 167: 379–383.
 100. Bitko V, Velazquez A, Yang L, Yang YC, Barik S. Transcriptional induction of multiple cytokines by human RSV requires activation of NF-kappa B and is inhibited by sodium salicylate and aspirin. *Virology* 1997; 232: 369–378.
 101. Sudo K, Yoshida K, Konno K, *et al.* Pharmacokinetics of a benzodithiin (RD3-0028) following aerosol treatment in rat. *Xenobiotica* 2002; 32: 19–27.
 102. Sudo K, Watanabe W, Konno K, *et al.* Efficacy of RD3-0028 aerosol treatment against RSV infection in immunosuppressed mice. *Antimicrob Agents Chemother* 1999; 43: 752–757.
 103. Wyde PR, Ambrose MW, Meyerson LR, Gilbert BE. The antiviral activity of SP-303, a natural polyphenolic polymer, against respiratory syncytial and parainfluenza type 3 viruses in cotton rats. *Antiviral Res* 1993; 20: 145–154.
 104. Barnard DL, Huffman JH, Meyerson LR, Sidwell RW. Mode of inhibition of RSV by a plant flavonoid, SP-303. *Chemotherapy* 1993; 39: 212–217.
 105. Douglas JL, Paris ML, Ho E, *et al.* Inhibition of respiratory syncytial virus fusion by the small molecule VP-14637 via specific interactions with F protein. *J Virol* 2003; 77: 5054–5064.
 106. Morton CJ, Cameron R, Lawrence LJ, *et al.* Structural characterization of respiratory syncytial virus fusion inhibitor escape mutants: homology model

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- of the F protein and a syncytium formation assay. *Virology* 2003; 311: 275–288.
107. Razinkov V, Huntley C, Ellestad G, Krishnamurthy G. RSV entry inhibitors block F-protein mediated fusion with model membranes. *Antiviral Res* 2002; 55: 189–200.
 108. Andries K, Moeremans M, Gevers T, et al. Substituted benzimidazoles with nanomolar activity against respiratory syncytial virus. *Antiviral Res* 2003; 60: 209–219.
 109. Wyde PR, Chetty SN, Timmerman P, Gilbert BE, Andries K. Short duration aerosols of JNJ 2408068 (R170591) administered prophylactically or therapeutically protect cotton rats from experimental respiratory syncytial virus infection. *Antiviral Res* 2003; 60: 221–231.
 110. Shigeta S, Mori S, Kodama E, Kodama J, Takahashi K, Yamase T. Broad spectrum anti-RNA virus activities of titanium and vanadium substituted polyoxometalates. *Antiviral Res* 2003; 58: 265–271.
 111. Ohki S, Liu JZ, Schaller J, Welliver RC. The compound D4EM inhibits respiratory syncytial virus fusion activity with epithelial cells. *Antiviral Res* 2003; 58: 115–124.
 112. Kimura K, Mori S, Tomita K, et al. Antiviral activity of NMSO3 against respiratory syncytial virus infection *in vitro* and *in vivo*. *Antiviral Res* 2000; 47: 41–51.
 113. Zamecnik P. Background of the antisense oligonucleotide approach to chemotherapy. *Antisense Nucleic Acid Drug Dev* 1997; 7: 199–202.
 114. Piroloa KF, Raita A, Sleerb LS, Changa EH. Antisense therapeutics: from theory to clinical practice. *Pharmacol Ther* 2003; 99: 55–77.
 115. Torrence PF. 2-5A-antisense chimeras: inhibitors of respiratory syncytial virus infection. *Curr Opin Mol Ther* 1999; 1: 307–315.
 116. Leaman DW, Longano FJ, Okicki JR, et al. Targeted therapy of respiratory syncytial virus in African green monkeys by intranasally administered 2-5A antisense. *Virology* 2002; 292: 70–77.
 117. Bitko V, Barik S. Phenotypic silencing of cytoplasmic genes using sequence-specific double-stranded short interfering RNA and its application in the reverse genetics of wild type negative-strand RNA viruses. *BMC Microbiol* 2001; 1: 34.
 118. Jacque JM, Triques K, Stevenson M. Modulation of HIV-1 replication by RNA interference. *Nature* 2002; 418: 435–438.
 119. Coburn GA, Cullen BR. Potent and specific inhibition of human immunodeficiency virus type 1 replication by RNA interference. *J Virol* 2002; 76: 9225–9231.
 120. Elbashir SM, Harborth J, Lendeckel W, Yalcin A, Weber K, Tuschl T. Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. *Nature* 2001; 411: 494–498.
 121. Ahlquist P. RNA-dependent RNA polymerases, viruses, and RNA silencing. *Science* 2002; 296: 1270–1273.
 122. Geiss G, Jin C, Cuo J, Bumgarner R, Katze MG, Sen GC. A comprehensive view of regulation of gene expression by double-stranded RNA-mediated cell signaling. *J Biol Chem* 2001; 276: 30178–30182.
 123. Byrd LG, Prince GA. Animal models of respiratory syncytial virus infection. *Clin Infect Dis* 1997; 25: 1363–1368.
 124. Sudo K, Watanabe W, Mori S, Konno K, Shigeta S, Yokota T. Mouse model of respiratory syncytial virus infection to evaluate antiviral activity *in vivo*. *Antivir Chem Chemother* 1999; 10: 135–139.
 125. Kimpen J. Experimental models for respiratory syncytial virus infections. *Rev Med Microbiol* 1996; 7: 115–122.
 126. Weltzin R, Traina-Dorge V, Soike K, et al. Intranasal monoclonal IgA antibody to RSV protects rhesus monkeys against upper and lower respiratory tract infection. *J Infect Dis* 1996; 174: 256–261.
 127. Leader S, Kohlhase K. Recent trends in severe respiratory syncytial virus (RSV) among US infants 1997 to 2000. *J Pediatr* 2003; 143: S127–S132.
 128. Maggon K. The ten billion dollar molecule. *Pharm Exec* 2003; 23: 60–68. (<http://www.pharmexec.com/pharmexec/content/contentDetail.jsp?id=75665>).
 129. Sellers LJ. Big pharma bails on anti-infective research. *Pharm Exec* 2003; 23: 22.
 130. Gallagher R. Vaccination undermined. *The Scientist* 2003; 17: 6.
 131. Institute of Medicine IOM USA Report. *Financing Vaccines in the 21st Century*. National Academic Press. 2003. (<http://www.nap.edu>)
 132. Beardsley TM. Adjusted agenda. AIDS activists accelerate alternative approaches. *Sci Am* 1990; 263: 30–32.
 133. Thompson D. Your money or their lives. Patient advocates are learning from AIDS activists how to work the system. *Time* 1992; 140: 66–68.
 134. Macilwain C. AIDS activists say basic research is underfunded. *Nature* 1993; 363: 388.
 135. Check E. Gene regulation: RNA to the rescue? *Nature* 2003; 425: 10–12.
 136. Dave RS, Pomerantz RJ. RNA interference: on the road to an alternate therapeutic strategy! *Rev Med Virol* 2003; 13: 373–385.
 137. Varmus H, Klausner R, Zerhouni E, Acharya T, Daar AS, Singer PA. Grand challenges in global health. *Science* 2003; 302: 398–399. (<http://www.grandchallengesgh.org>)

138. Medicine for Malaria Venture (mmv.org); Global Alliance for TB Drug Development (tballiance.org); Global Fund to fight AIDS, Tuberculosis and Malaria (theglobalfund.org); Drugs for Neglected Diseases Initiative (dndi.org); Global Alliance for Vaccines and Immunization GAVI at WHO

(who.int); International AIDS Vaccine Initiative (iavi.org); Global Forum for Health Research (globalforumhealth.org); Bill and Melinda Gates Foundation (gatesfoundation.org/GlobalHealth/InfectiousDiseases/). All addresses follow <http://www>.